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Estimation of sensitivity and specificity of antinuclear antibody by automated indirect immunofluorescence and enzyme-linked immunoassay

Mohammadreza Sheikh Sajjadieh [1*], Ali Ajami [1,2], Leila Haghshenas [3]

1. Department of Immunology, Nobel Medical Laboratory, Isfahan, Iran
2. Medical Diagnostic Laboratory of Isfahan Province Health Center, Isfahan University of Medical Science, Isfahan, Iran
3. Department of Clinical Bioinformatics, Harvard Medical School, Boston MA, USA

* Correspondence: Mohammadreza Sheikh Sajjadieh. Department of Immunology, Nobel Medical Laboratory, Isfahan, Iran.
Tel: +989138345076; Email: mohammad_esfahan@yahoo.com

Abstract

Background: Immunofluorescence and serology analysis are the most common laboratory methods for diagnosing antinuclear antibodies in autoimmune diseases and are paramount for screening and therapeutic purposes. This study aims to estimate the sensitivity and specificity of antinuclear antibodies measured by automated indirect immunofluorescence and enzyme-linked immunoassay in patients at risk for autoimmune diseases.

Methods: Serum antinuclear antibodies in 3020 patients suspected of autoimmune diseases at Nobel Medical Laboratory, Esfahan, IRAN, were measured from 2017 until 2020 with automated indirect immunofluorescence and enzyme-linked immune assay methods. The sensitivity, specificity, prevalence, positive and negative predictive value, and likelihood ratio were calculated for each technique. In addition, the receiver operating characteristic curve (ROC) was analysed as a statistical method for assessing the diagnostic accuracy of these tests.

Results: The immunofluorescence method demonstrated low sensitivity and high specificity compared with the enzyme-linked immunoassay. For the automated indirect immunofluorescence method, sensitivity and specificity were 88% and 62%, respectively, whereas this number for the ELISA method was determined as 89.6% and 28.5 %, respectively.

Conclusion: It is crucial to choose a suitable method for detecting autoantibodies for diagnostic purposes. ANA analysis by a sensitive test, such as an enzyme-linked immunoassay, should be used for screening. In contrast, a highly specific test, such as an indirect immunofluorescence assay, should be used to confirm the result and monitor dynamic treatment.

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Introduction

Systemic Lupus Erythematosus (SLE) includes a heterogeneous group of autoimmune disorders with different clinical manifestations, such as musculoskeletal, cutaneous, renal, neuropsychiatric, pulmonary, cardiac and hematologic presentations, and is more frequent in women. Despite differences in structure and statistical performance, the European League against Rheumatism and American College of Rheumatology and Systemic Lupus International Collaborating Centers criteria agree on the importance of both immunological and clinical findings (1). The immunological findings include the detection of autoantibodies against nuclear proteins, anti-dsDNA, anti-phospholipid or other components of human cells such as native DNA, nucleosome, Smith antigen (SM), Sjogren syndrome antigen (SS-A, SS-B) and low concentrations of serum complement and positive Coombs test without hemolysis.

Anti-nuclear antibodies (ANA) play a crucial role in diagnosing autoimmune diseases. Their levels are measured through indirect immunofluorescence assay (IIFA) or serology equivalent assay (2), such as enzyme-linked immune assay (ELISA). Notably, sensitivity and specificity are related to the diagnostic techniques or platform used (3). Sensitivity and specificity demonstrate the diagnostic accuracy of a test. They are indicators of the test's ability to distinguish between disease and the absence of disease at a chosen cut-off. Several terms are commonly used along with the description of sensitivity, specificity and accuracy, including true positive (TP), true negative (TN), false negative (FN), and false positive (FP).

Optimisation and standardisation of the ANA test are essential because concurrent and proper diagnostic methods with better specificity and sensitivity are necessary in clinical practice. Therefore, we decided to estimate the sensitivity and specificity of the ANA test in patients with possible autoimmune diseases. This was realised using ELISA and a unique immune fluorescence image processing system, which is only available at our facility in the region.

Methods

We included 3020 patients with a suspected autoimmune disease from July 2017 to August 2020 and tested them at the Department of Immunology of the Nobel medical laboratory in Esfahan. Since the research did not involve a student from a research university or a research proposal, we have not obtained approval from the ethical committee. We used an automated IIF processor and analysed 2300 serum samples following the kit's protocol (Aklides, Germany). Seven hundred twenty serum samples were analysed using an ELISA commercial kit (Orgentec kit and Alegria system). Aklides is a semiautomatic IIF processor for reading prepared ANA-IIF on the HEp-2 cell with commercial test kits (Generic Assays GmbH Dahlewitz, Berlin, Germany). The Aklides system comprises a motorised scanning stage and a fully automated fluorescence microscope (Olympus IX81, Olympus Corporation). This uniquely designed software system (Aklides) employs mathematical algorithms for pattern recognition and light intensity determination. Sera samples with a titer of $\geqslant$1:160 and a light intensity value of $\geqslant$100 were considered positive. Statistical analysis was performed with SPSS 11.

The graphical ROC curve was created by plotting sensitivity (True positive rate) on the y-axis against 1-specificity (False positive rate) on the x-axis.

Results

The majority of the patients were women (65%). In both group assays, TP, TN, FP, and FN were estimated according to the presence or absence of clinical symptoms such as joint swelling and other measurable laboratory tests, including C3, C4, CRP, and ESR (Data not shown).

The results of the indirect immunofluorescence method (Table 1) and the ELISA method (Table 2) regarding TP, TN, FP, and FN values were recorded.

The sensitivity and specificity for automated indirect immunofluorescence were estimated at 88% and 62 %, whereas the number for the enzyme-linked immune assay was 89.6% and 28.5% (Table 3). The receiver operating characteristic curve (ROC) was analysed to provide a statistical method for assessing the diagnostic accuracy of these tests (Figure 1). As can be seen in Table 3, other statistical parameters such as prevalence, positive and negative predictive value, and likelihood ratio of ANA tests are used by both methods.

Table 1. Description result obtain from indirect immunofluorescence method.

Outcome of the diagnostic test	Condition as determined by IF		
	Positive	Negative	Total
Positive	TP=1320	FP=304	TP+FP=1624
Negative	FN=180	TN=496	FN + TN=676
total	1500	800	2300

TP: True Positive, FP: False Positive, FN: False Negative, TN: True Negative.

Table 2. Description result obtained from ELISA method.

Outcome of the diagnostic test	Condition as determined by ELISA		
	Positive	Negative	Total
Positive	TP=496	FP=120	TP+FP=616
Negative	FN=56	TN=48	FN + TN=104
Total	552	168	720

TP: True Positive, FP: False positive, FN: False Negative, TN: True Negative

Table 3. Contingency table of automated and manual results.

Method	ELISA	IF
Number of participates	720	2300
Sensitivity %	89.86	88.0
Specificity %	28.57	62.0
Positive predictive value %	80.52	81.28
Negative predictive value %	46.16	73.37
Likelihood ratio (+)	1.26	2.32
Likelihood ratio (-)	0.35	0.19
Prevalence %	76.67	65.22

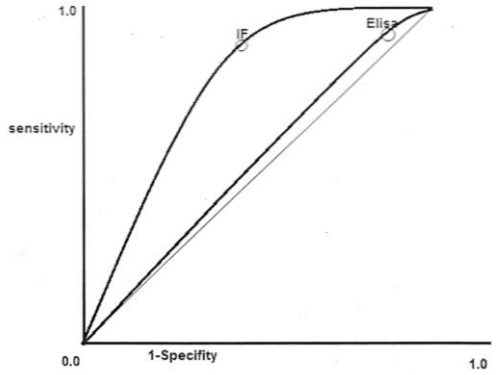

Figure 1. Receiver operating characteristic curve (ROC) analysis of ELISA and indirect immunofluorescence.

Discussion

ANA testing is essential to diagnosing autoimmune disease; it is a cost-effective method in screening, prognosis and therapeutic targets. However, it should be noted that the autoantibody determination technique's sensitivity, specificity, and clinical associations can vary according to the diagnostic methods or platforms used. Generally, due to the variety of cut-offs of each technique in clinical practice, clinicians are challenged to perform concurrently with IIF and ELISA due to the sensitivity and specificity of a test. In this study, we aimed to investigate appropriate sensitivity, specificity, and positive and negative predictive values. Moreover, we created a receiver operating characteristic (ROC) with the area under the curve (AUC) for both present ELISA and IIF kits. These two kits are generally used to diagnose autoimmune diseases based on ANA according to clinical history and physical examinations (4). Although no ANA test with 100 % sensitivity and specificity exists, a test can be very specific without high sensitivity or very sensitive without high specificity. Both factors are equally important, and a desirable test should have high sensitivity and specificity.

We compared the ANA results measured by ELISA with those obtained through automated IIF. The serology method has high sensitivity (89.6 %) and low specificity (28.57%). In contrast, the results of IIF had low sensitivity (88%) and high specificity (62%). The ROC curve (Figure 1) demonstrated that Aklides was a suitable method for the detection of ANA with an appropriate diagnostic accuracy because it had a ROC curve in the upper left triangle as shown in figure (AUC IIF>AUC ELISA). High-sensitivity tests are more suitable for screening and detecting many patients suspected of autoimmune disease. In contrast, an appropriate test for confirmation of diagnosis and follow-up during treatment should have more specificity.

In agreement with others, we detected a higher prevalence of autoimmune diseases in females (65%) than in males (35%). This can be attributed to exogenous or endogenous hormones, such as steroid hormones, including estrogens and androgens, which are known to generate autoantibodies (5).

In contrast with our study, a similar study demonstrated that the detection of ANA by ELISA had low sensitivity and high specificity compared with IIF (6). Another published article recently showed that the ELISA method had more sensitivity and specificity than the IIF method (7). This can be attributed to different factors. For instance, the ELISA method is a solid-phase immune assay that includes 17 ANA-targeted recombinant antigens (7). In contrast, HEP 2 cells in the IIF method allow a visible antibody-antigen reaction, especially with cellular protein, which is visualised as nuclear or cytoplasmic. A cytoplasmic reaction is a false positive result in the ANA test, determined as a positive result by ELISA.

In agreement with our study, Clemens Dario et al. in Germany, which detected ANA by two automatic systems (Aklides and Helios), obtained sensitivity and specificity similar to our result (8). Therefore, automated IIF can reliably differentiate positive from negative and weak positive samples.

Conclusion

In conclusion, generally, ANA measurement by a highly sensitive test such as ELISA should be used for SLE screening purposes to identify most cases. In contrast, detecting ANA with highly specific tests, such as IIF, should be used to confirm the diagnosis and monitoring purposes during treatment.

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Ethical statement

The authors acknowledge that this article has not been published in any other journal or scientific institution, there is no conflict of interest, and all the ethical considerations of human experimentation have been properly performed. There was no need for cell culture, obtaining informed consent, or similar permissions.

Conflicts of interest

The authors declare that they have no conflicts of interest.

Author contributions

The first and second authors contributed equally to writing, conducting experiments, and preparing the material. The third author participated in data extraction and quality assessment, manuscript review, final approval, and final version preparation.

References

1. Fava A, Petri M. Systemic lupus erythematosus: Diagnosis and clinical management. J Autoimmun. 2019;96:1-13. [View at Publisher] [DOI] [PMID] [Google Scholar]
2. Aringer M, Petri M. New classification criteria for systemic lupus erythematosus. Curr Opin Rheumatol. 2020;32(6):590-6. [View at Publisher] [DOI] [PMID] [Google Scholar]
3. Jamshidi AR, Tehrani Banihashemi A, Roknsharifi Sh. Estimating the prevalence and disease characteristics of rheumatoid arthritis in Tehran: A WHO -ILAR COPCORD Study (from Iran COPCORD study, Urban Study stage 1). Med J Islam Repub Iran. 2014;28: 93. [View at Publisher] [PMID] [Google Scholar]
4. Agmon-Levin N, Damoiseaux J, Kallenberg C, Sack U, Witte T. International recommendations for the assessment of autoantibodies to cellular antigens referred to as anti-nuclear antibodies. Ann Rheum Dis. 2014;73:17-23. [View at Publisher] [DOI] [PMID] [Google Scholar]
5. 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. [View at Publisher] [DOI] [PMID] [Google Scholar]
6. Sumanth Kumar GLS, Chaudhury A, Verma A, Kumar Bhattaram S. Comparison of enzyme linked immunosorbent assay (ELISA) with indirect immunofluorescence for detection of anti-nuclear antibody. The Journal of Clinical and Scientific Research. 2014;3(4):237-42. [View at Publisher] [Google Scholar]
7. Alsaed OS, Alamlih LI, Al-Radideh O, Chandra P, Alemadi S, Al-Allaf AW. Clinical utility of ANA-ELISA vs ANA-immunofluorescence in connective tissue diseases. Sci Rep. 2021;11(1):8229. [View at Publisher] [DOI] [PMID] [Google Scholar]
8. Loock CD, Egerer K, Feist E, Burmester GR. Automated evaluation of ANA under real-life conditions. RMD Open. 2017;3(1):e000409. [View at Publisher] [DOI] [PMID] [Google Scholar]

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