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Evaluation of Diagnostic Significance and Cost Effectiveness of ELISA and IFA for the Diagnosis of Autoimmune Disorders

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Abstract

Background: The presence of antinuclear antibodies (ANA) is a hallmark of autoimmune diseases. As Clinicopathological classification of autoimmune diseases is difficult without laboratory support, laboratory testing is of helps in diagnosis, treatment, prognosis, and prediction of the pathological changes by disease activity. Although different tests are available for ANA detection enzyme linked immunosorbent assay (ELISA) is the mainstay of diagnosis in most routine laboratories. Indirect immunofluorescence antinuclear antibody test (IFA) though currently the "gold standard" it is not widely practiced. Most studies have used Hep2 cells for the detection of autoantibodies by IFA. However Hep 2000 Ro is superior compared to Hep 2 which lacks capability of detecting some autoantibodies like Ro antibodies. Hence, this study was undertaken to compare the diagnostic value and cost effectiveness of ANA pattern, ELISA with profile testing for patients suspected to have autoimmune disorders.

Results: In the present study we observed high prevalence of autoimmune diseases in females (75.82%).ANA-ELISA in criteria matched cases with respect to ANA-IFA had a low sensitivity (59% versus 80%), higher specificity (84% versus 70%). Statistical analysis of ELISA and IFA with respect to ANA Profile showed a very less sensitivity by ELISA over IFA (51% *vs.* 78%) and equal specificity (70-72%) in 142 criteria matched cases.

Conclusions: Statistically significant differences between ELISA and IFA infers IFA-ANA is a very appropriate method for screening purposes also IFA have capability of finding anti-mitochondrial and other cytoplasmic antibodies, which is not possible with ELISA.

Keywords: ANA; ELISA; IFA; ANA profile

Abbreviations SLE: Systemic Lupus Erythematosus; CTD: Connective Tissue Diseases; MCTD: Mixed Connective Tissue Diseases; ANA: Anti-Nuclear Antibody; IFA: Indirect Immunofluorescence Antinuclear Antibody; ELISA: Enzyme Linked Immunosorbent Assay

Introduction

Autoimmune diseases are chronic in nature requiring a lifetime treatment. Autoimmune diseases are frequent disease complexes, affecting 5% to 7% of the population. They are the third common cause for mortality. Autoimmune diseases are chronic in nature requiring a lifetime of care. The presence of antinuclear antibodies (ANA) is a hallmark of autoimmune diseases. As clinico-pathological classification of autoimmune diseases is difficult without laboratory support, laboratory testing helps in diagnosis, treatment, prognosis, and predict pathological changes by disease activity.

The practice of evidence-based medicine has emphasized the need for specific guidelines in clinical and laboratory diagnosis of autoimmune diseases. Thus leading to uncontrolled emergence of new methods and increased expenditure of economic resources for the assay of autoantibodies. Accepting that in daily life, many tests are requested for patients with or without manifestation of autoimmune process, the laboratory needs adequate and reliable screening tests that are relatively cost-effective in comparison to the third generation tests like line immunoassay.

Although different tests are available for ANA detection like Gel precipitation assays, Passive hemagglutination(PHA), Multiplex Immunoassay(MIA), Dot blot, Line blot Immunoassay, Multiplex Bead based Assay, Microarray based assays enzyme linked immunosorbent assay (ELISA) is the mainstay of diagnosis in most routine laboratories. Indirect immunofluorescence antinuclear antibody test (IFA) though currently the "gold standard" it is not widely practiced [1]. Most studies have used Hep2 cells for the detection of autoantibodies by IFA. However Hep 2000 Ro is superior compared to Hep 2 which lacks capability of detecting some autoantibodies like Ro antibodies.

Various studies on concordance of ANA-IFA and ANA-ELISA results in autoantibody testing have been reported previously. However, the correlations between ANA results by these two methods and the presence of specific autoantibody by ANA profile in these patients were not described in these studies. However no such study has been published till date from the State of Kerala.

Thus this study was designed to evaluate the diagnostic performance and usefulness of ANA testing by ELISA compared with Hep 2000 Ro IFA test. Also results of this study would provide for reference database for future studies in our community on the best diagnostic algorithm that would accurately predict specific autoimmune disorders. Hence, this study was undertaken to compare

the diagnostic value and cost effectiveness of ANA pattern, ELISA with profile testing for patients suspected to have autoimmune disorders.

Methods

This study was conducted at Pushpagiri institute of medical sciences and research Center, Thiruvalla from January 2013 to November 2014. Pregnant women, children (less than 14 years) and known cases of Rheumatoid arthritis were excluded from the study.

All the samples requested for ANA testing were preceded by performing both IFA and ELISA. All the cases were categorized into four broad groups depending upon the presence of clinical reference criteria as criteria matched cases and screening cases.

a. All clinically suspected cases

b. Criteria matched cases which were further classified as

1. Individual Criteria matched CTDs (connective tissue diseases) and

2. Other criteria matched rare autoimmune diseases

Of the 454 patient serum samples collected during the overall two year period, 182 patients (40%) presented with features of characteristic diagnostic criteria of autoimmune disease (criteria matched cases). The remaining 272 (60%) patients presented with nonspecific symptoms. Autoimmune disorders were a part of the differential diagnosis in these cases. ANA ELISA and IFA were requested as a screening test in these patients to rule out the possibility of autoimmune diseases. Of the total 182 patients, 142 (78%) had clinical features strongly suggestive of a CTD as per the standard clinical criteria published for each disorder. These were called as criteria matched CTD cases. The remaining 40 (22%) cases included cases of other rare autoimmune disorders like myositis, autoimmune hepatitis, nephritis, drug induced lupus, evolving RA, cutaneous small vessel vasculitis, and Idiopathic Thrombocytopenic purpura (ITP).

IFA-ANA test

We used immunofluorescence kit ANA Hep-2000^{R} test system Immunoconcepts, Costa Brava 30, Barcelona, Spain and sera processed at 1:80 at dilution. IFA was performed according to the manufacturer's protocol. Negative and positive controls were used to check accuracy of every run. The slides were read with a fluorescence microscope (Carl Ziess primostar iLED equipped with a 30-W HAL 6 halogen lamp). The fluorescence intensity was interpretated +1 to +3 relative to the intensity of a negative and a positive control (+3).

ELISA

All the sera processed for ELISA using a commercially available ELISA kit (Lilac Organtec diagnostics, Germany) at 1:100 dilutions. These kits comprise strip coated with affinity purified 26 antigens.

All serum samples were screened with IFA and ELISA for total ANA and samples of criteria matched cases selected by cluster randomization were tested further by ANA profile to determine the specific autoantibodies.

ANA profile

ANA profile (Medizinische Laboratory diagnostic, deutschland) was performed and presence of specific ANA was detected by automated evaluation with Euro line scanning device (EURO LINE SCANNED) with specialized software after calibration and validation according to manufacturer's recommendations. The result was presented graphically, and the reaction intensity, automatically measured by the software was objectively interpreted and semi quantitatively assessed by the program as negative (-), borderline (±), slightly positive (+), moderately positive (++) and strongly positive (+++) for ANA. Digitalized images of the immunoblot were saved and archived for re-analysis if necessary.

Statistical analysis

Agreement with respect to the final result between different methods was assessed by κ (kappa) coefficient of Cohen by Vassar Stats calculator. A Kappa statistic <0.2 was considered to indicate a "poor" strength of agreement; 0.21-0.40 was "fair," 0.41–0.60 was "moderate," 0.61-0.80 was "good,". All the other data analyses were performed using software Epi info, release 20.0. Data for sensitivity and specificity were analyzed with statistical software MedCalc, Version 8.1.0.0 (MedCalc Corp., Mariakerke, Belgium).

Results

Of the 454 patient serum samples collected during the overall two year period, 182 patients (40%) presented with features of characteristic diagnostic criteria of autoimmune disease (criteria matched cases). The remaining 272 (60%) patients presented with nonspecific symptoms (Figure 1).

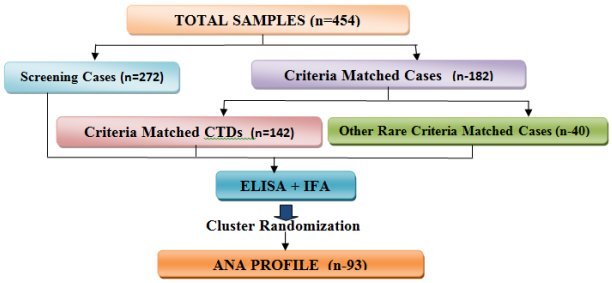

Figure 1: Total 454 patient serum samples collected during the overall two year period, 182 patients (40%) presented with features of characteristic diagnostic criteria of autoimmune disease (criteria matched cases). The remaining 272 (60%) patients presented with non-specific symptoms.

Autoimmune disorders were a part of the differential diagnosis in these cases. ANA ELISA and IFA were requested as a screening test in these patients to rule out the possibility of autoimmune diseases of the total 182 patients, 142 (78%) had clinical features strongly suggestive of a CTD as per the standard clinical criteria published for each disorder [2]. These were called as criteria matched CTD cases. The remaining 40 (22%) cases included cases of other rare autoimmune disorders like myositis, autoimmune hepatitis, nephritis, drug induced lupus, cutaneous small vessel vasculitis, and Idiopathic Thrombocytopenic purpura (ITP).

The study population consisted of 319 (67.87%) females and 151 (32.13%) males of 454 clinically suspected autoimmune diseases. The likely explanation is exogenous or endogenous hormones like steroid hormones, including estrogens and androgens, are known to influence antibody production and immune cell proliferation [3]. Thus, hormones can amplify or inhibit the immune response.

In present study majority of the criteria matched cases (n=44, 24.18%) were from patients in the age group between 26-35 years. High number of cases in the early age group may be due to changes in the environmental conditions which can influence or affect genetic factors predisposing the person to autoimmune diseases [4].

Statistical significance of the ANA ELISA and IFA results were compared between all cases, criteria matched cases, criteria matched CTDs and other rare criteria matched cases (Tables 1 and 2) and their agreement by Kappa analysis is elaborated in Table 2.

ELISA Results-All cases (n=454)	IFA Results-All cases (n=454)	
	POSITIVE	NEGATIVE
POSITIVE	57 (12.6%)	27 (5.9%)
NEGATIVE	40 (8.8%)	330 (71.8%)

Table 1: Comparison of ELISA *vs.* IFA in All cases (n=454).

PARAMETERS	ELISA	IFA
All cases (n=454)		
Sensitivity	58% (CI-48.7%-68.6%)	68% (CI-56.8-77.6%)
Specificity	92.4% (CI-88.5%-94.2%)	89% (CI-86.6%-92.7%)
Kappa statistics & Strength of association	0.538 & Moderate (CI 0.44-0.635)	
Criteria matched cases (n=182)		
Sensitivity	59% (CI-49.2%-69.8%)	80% (CI-68%-88.9%)
Specificity	84% (CI-75.7%-91.21%)	70% (CI-57.9%-76.1%)
Kappa statistics & Strength of association	0.519 & Moderate (CI 0.39-0.64)	
Criteria matched CTD (n=142)		
Sensitivity	62% (CI-50.1%-73.2%)	84% (CI-71.7%-92.2%)
Specificity	86.7% (CI-76.4%-93.7%)	70% (CI-57.1%-77.9%)
Kappa statistics & Strength of association		0.484 & Moderate (CI 0.35-0.62)
Other rare autoimmune diseases (n=40)		
Sensitivity	52% (CI-21.6%-77.1%)	75% (CI-43%-94.5%)
Specificity	86.9% (CI-66.4%-97.2%)	72% (CI-51%-87%)
Kappa statistics & Strength of association		0.415 & Moderate (CI 0.136-0.69)

Table 2: Statistical significance of ELISA and IFA in respect to each other.

Overall ANA-ELISA in criteria matched cases with respect to ANA-IFA (with the reference range of 1:80) had a low sensitivity (59% *vs.* 80%), higher specificity (84% *vs.* 70%). In criteria matched CTD similar results were obtained: low sensitivity (62% *vs.* 84%), higher specificity (86.7% *vs.* 70%). ELISA was comparatively less sensitive than IFA for other rare autoimmune diseases (52% *vs.* 75%) but specificity was high for ELISA over IFA (86.9% *vs.* 72%).

IFA patterns

Predominant IFA patterns obtained by IFA were homogenous, speckled, nucleolar and centromere, SSA and cytoplasmic (Figures 2 and 3).The speckled patterns (39.2%) was the most common ANA pattern seen in positive patients followed by homogenous 26.3%, cytoplasmic 15%, SSA 5%, centromere 3% and nucleolar 4%.

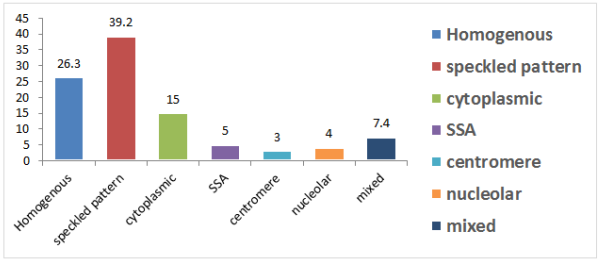

Figure 2: IFA Patterns.

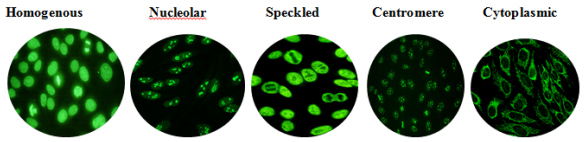

Figure 3: ELISA vs. IFA Patterns.

ELISA *vs.* IFA pattern

Comparison of ELISA with IFA patterns was done to assess whether there was any association between negative ELISA and the obtained IFA pattern. The comparison of IFA and ELISA in criteria matched systemic lupus erythematosus (SLE) patients, we observed the most commonly missed patterns by ELISA (38%) was cytoplasmic followed by homogenous and speckled [5]. Similarly in case of scleroderma, ELISA was reported negative in 37.5% of cases where IFA showed speckled, nucleolar and homogenous patterns. But a point to note is that all patients with centromere pattern were picked up by ELISA. In Sjogren's syndrome cases three patients showed SSA pattern out of which, two were negative by ELISA. In Mixed connective tissue diseases (MCTD), total six cases were negative by ELISA while showing homogenous and speckled patterns on IFA. Thus in total ELISA was negative in 37.5% of confirmed cases while IFA was positive.

ANA profile

Specific antibodies associated with disease can be characterized using the ANA profile [6]. Accordingly, ANA profiling was done for 93 samples, which were randomly selected out of the 182 criteria matched cases (Table 3). Among the randomly chosen 93 criteria matched cases 41 (44.1%) were detected positive for ANA profile by either a single or a combination of two or more specific autoantibodies.

ANA Profile Positive (n=41)		**ANA Profile Negative (n=52)**		
IFA Positive (n=32)	IFA Negative (n=9)	IFA Positive (n=18)	IFA Negative (n=34)	
17	4	7	7	ELISA Positive (n=35)
15	5	11	27	ELISA Negative (n=58)

Table 3: Comparison of ELISA, IFA, and ANA PROFILE in criteria matched cases

Individually ELISA and IFA results were compared with that of ANA profile. Statistical analysis and Kappa analysis of ELISA *vs.* ANA profile and IFA *vs.* ANA profile was carried out (Table 4).The comparison of IFA, ELISA with profile in 93 criteria matched cases where all the three tests were performed showed that 17 patients were positive and 27 were negative by all the three tests. Fifteen ELISA negative cases were positive by both IFA and profile but seven patients who were positive by ELISA were negative by IFA and ANA profile. Comparison of ELISA and ANA profile in criteria matched cases showed both positive in 21 (22.6%) cases and both negative in 38 cases (41%).

Parameters	**Criteria matched cases (n=93)**		**Criteria matched Common CTD(n=71)**		**Criteria matched other rare diseases (n=22)**	
	ELISA	IFA	ELISA	IFA	ELISA	IFA
Kappa statistics	0.24	0.424	0.31	0.462	-0.22	0.28
Strength of association	Fair (CI 0.05-0.44)	Moderate (CI 0.25-0.61)	Fair (CI 0.09-0.51)	Moderate (CI 0.26-0.67)	Worse (CI 0.42-0.55)	Fair (CI 0.03-0.55)

Table 4: Agreement between ELISA, IFA & ANA PROFILE.

Twenty ANA profile positives were negative by ELISA. Overall four IFA negatives were positive by ELISA and ANA profile while eleven IFA positives were negative by ANA profile and ELISA. Statistical analysis of ELISA and IFA in respect to ANA profile showed a very less sensitivity by ELISA over IFA (51% *vs.* 78%), (55% *vs.* 79%) in criteria matched cases, criteria matched CTD respectively. None of the other rare criteria matched cases were positive by ELISA whereas IFA showed 100% sensitivity. Both ELISA and IFA showed equal specificity (70-72%) in criteria matched cases, criteria matched CTD while in other rare criteria matched patients both ELISA and IFA were less specific (68% *vs.* 60%).Over all IFA showed moderate agreement with kappa values between 0.41-0.6 for CTD, criteria matched cases but agreement was fair with 0.28 kappa for rare disease (Table 4). ELISA had fair agreement with kappa value of 0.24*0.31 for criteria matched CTD patients respectively. But it had worse agreement for other rare criteria matched autoimmune diseases

ANA IFA pattern *vs.* ANA profile antibodies

An association between individual antibodies (ANA PROFILE) and IFA patterns was determined in all 93 cases (Table 5).

IFA PATTERN	**ANTIBODIES OBSERVED**
Centromere (n=2)	centromere B, Pm Scl, nucleosomes
Cytoplasmic (n=9)	Ro 52, dsDNA, Sn RNP, Jo 1, SSA, M2, Histone, SSA, Sm
Homogenous (n=10)	RNP, dsDNA, PCNA
Nucleolar (n=3)	Histones, SS A
Speckled (n=23)	dsDNA, histones, SSA, nucleosomes, Ku, Mi 2 ab, PCNA, RNP, Sm, Ro-52, Histones, Pm-Scl
SSA (n=2)	SSA, Ro 52, dsDNA
Negative (n=9)	PM Scl, dsDNA, Ribosomal P protein, Scl-70, Ro 52, SSA, Sm, RNP, Sm, PCNA

Table 5: IFA and ANA profile in criteria matched cases (n=93).

IFA patterns were compared with Profile results for various combinations of specific autoantibodies. Out of 93 criteria matched patients total 50 cases were positive by IFA. Among these 50 cases 32 (64%) cases were profile positive. In these 32 cases two patients with centromere pattern showed antibodies to CENP, Pm-Scl, nucleosomes by profile. In ten patients with the homogenous pattern four showed specific antibodies to RNP, dsDNA and PCNA. In patients with nucleolar pattern (n=3) two patients are positive for antibodies to Histones, SSA by profile. In patients with the speckled pattern (n=23), 17 showed antibodies to dsDNA, PCNA, RNP by profile. Along with these antibodies most of the cases also showed other antibodies like histones, SSA, nucleosomes, Ku, Mi2, Sm, Ro-52 and Pm-Scl. All patients with SSA (n=2) pattern by IFA were positive by profile with specific antibodies to SSA, Ro 52, dsDNA. Of the nine patients with cytoplasmic pattern six showed antibodies to M2, histones, SSA, Sm, dsDNA, Pm Scl, nucleosomes.

Discussion

Testing for ANAs is the initial step in the evaluation of patients with manifestations suggestive of autoimmune disorders. The purpose of this study was to evaluate the diagnostic value, cost effectiveness and usefulness of the available screening methods. In developing countries like India, there is an urgent need for screening tests that are relatively cheaper and reliable in comparison to the third generation test like line immunoassays (ANA profile).

In the present study, we observed high prevalence of autoimmune diseases in females (75.82%) than in males (24.18%). The likely explanation is exogenous or endogenous hormones like steroid hormones, including estrogens and androgens, are known to influence antibody production and immune cell proliferation [2]. Thus, hormones can amplify or inhibit the immune response. Estradiol binds to receptors on T and B lymphocytes, and increase activation and survival of those cells, thus favoring prolonged immune responses leading to elevated antibody response, while men often develop more severe inflammation [3].

In present study, majority of the criteria matched cases (n=44, 24.18%) were from patients in the age group between 26-35 years. High number of cases in the early age group may be due to changes in the environmental conditions which can influence or affect genetic factors predisposing the person to autoimmune diseases [5].

Overall ANA-ELISA with respect to ANA-IFA had a low sensitivity higher specificity in all the scenarios (Table 2). Studies conducted by Richard et al. [5] showed high specificity like in our study but, sensitivity is same for both test and which contrasts with findings from our study. Further, our results are in agreement with the studies conducted in clinically suspected autoimmune diseases patients by Sumanth, et al. at Tirupathi and Priyadarshini et al. at Chennai [6,7].

Difference in the statistical values of ELISA and IFA from present study to other studies 6 and 7 were probably due to difference in the inclusion criteria, study design, substrate used by IFA (HEp 2000Ro in our study *vs.* only HEp 2 in other studies) and difference in sample dilution used for testing by IFA and kits from different manufacturer by ELISA.

The final assessments of the clinically suspected 454 samples ANA results obtained from ELISA and IFA were compared by Kappa analysis. The majority of the results were in agreement (n=387, 85.2%), whereas 15%; n=67 yielded divergent results. The lack of agreement between test methods may reflect differences in the array of antigens present in the various assays. Kappa values results exhibited moderate agreement, with a Cohen's Kappa value of 0.497 (95% CI=0.39.*0.59) in clinically suspected cases. The IFA and ELISA results for criteria matched patients exhibited fair agreement with kappa value of 0.368 (95% CI=0.26-0.5), and the agreement for criteria matched CTD and other rare disease were fair with kappa values 0.397, 0.357 respectively (Table 2).

A study conducted in Bulgaria by Murdjeva et al. 8 in 2011, showed very good agreement between the ELISA and IFA (Kappa-0.890) which was high when compared to the present study. The difference in the strength of agreement may be due to difference in the IFA serum dilution cut off titre (1:40 *vs.* 1:80). The study by showed less agreement (kappa 0.30) which may be due to high IFA serum dilution cut off (1:160) and less number (n=7) of antigens in the kit used for ELISA in compared to our (n=26) study.

The most probable reason for the different statistical results and ELISA negative with definitive IFA patterns may be due to the fact that the ELISA test formats use a cocktail of 10-14 antigens depending on the commercial kit used. The antigens used may be native or recombinant. The amounts of nuclear antigens may not be constant because the affinity of each antigen for the solid phase is different [8].

On the other hand, IFA test formats use HEp-2 cells which contain high concentration of nuclear and cytoplasmic antigens which results in higher sensitivity. Thus, it is understandable that ELISA with its limited set of antigens may fail to detect certain antibodies which can be detected by IFA. Alternatively, it could be because ELISA could not detect low positives. The other likely explanation for different statistics may be due to the limited number of antigens used in the ELISA which may not reflect the autoantibody profile of the local population [9].

There may also be racial and/or ethnic differences in the autoantibody patterns found in different population groups10. So kits manufactured from other places may not reflect the autoantibodies prevalent in the local community [10,11].

Thus, the statistical significance of ELISA and IFA in respect to profile showed poor sensitivity by ELISA in all the scenarios. ELISA was not able to detect single case of rare criteria matched autoimmune disease. Whereas, IFA showed very good sensitivity and good specificity in all the three scenarios. Statistical significance of IFA, ELISA in respective of profile showed moderate significance by IFA indicating IFA is very reliable test for screening the autoimmune disease.

Eighteen out of 50 samples did not show any banding by Profile immunoassay though they were positive by IFA. Probably these serum samples had some antibodies other than the most common ones coated on the strips used in profile assay. The same issue that is IFA positive profile negative was addressed in other studies in 201011 and 20138 reported 48% [12], 17.2% [13] respectively. Hence, though by line assay we could differentiate between different ANAs, it may sometimes miss the detection of rare ANAs. Some kits offer 13 antigens, while some offer 15 and others 17 in their ANA profile kit. We used the immunoassay strip having 17 antigens coated on it. It is therefore likely that in the 18 profile negative cases studied here, the serum probably had some antibodies other than the most commonly encountered 14 antigens.

Clinical diagnosis of these 18 cases revealed that nine of these cases were rare autoimmune diseases. As only common and significant autoantibodies are tested on the line immunoassay, these negative ANA profile but positive IFA patients should be followed up to truly understand the significance of these positive IFA results.

On the contrary, nine sera positive for line immunoassay were negative by ANA-IIF. Two of these sera showed SSA/Ro-52 positivity, antibodies to PCNA in 2 cases, Sm in 2 cases and in rest of the cases showed positivity for Ribosomal P protein, Scl 70,Pm Scl, ds DNA. A similar observation was noted by Vos et al. [12] and Hoffman et al. [13]. Considering the fact that we used Hep2000, antibodies to SSA should have been picked up by our study. Renata Baronaite et al. [9] also used Hep2000 similar to our study and he also found a similar lacking of detection of antibodies to SSA in some cases. This is explained by the fact that line immunoassay is more sensitive in detecting antibodies to SSA/Ro-52 than ANA-IFA even when Hep-2000 cells are used [14]. HEp-2000 cells as a substrate for IFA ANA testing only improves upon the insufficient sensitivity of SSA/Ro antibodies; this method still fails to detect this antibodies.

Although some IFA patterns strongly suggest distinct specificities, additional tests are requested to demonstrate antibody reactivities against specific nuclear and cytoplasmic antigens [15]. These tests are used to either support the diagnosis (disease specificity) or to identify subsets of patterns that are prone to particular disease manifestation (as a prognostic marker) [16]. Further, the results of the test can be used in patients with a wide differential diagnosis, so that the results of the tests may exclude systemic autoimmunity (negative predictive value) or may assist the diagnostic process by meeting the diagnostic criteria of a particular autoimmune disease (disease specificity).

Evaluation of clinical diagnoses obtained from the history and medical records together with IFA and ANA profile results showed IFA test alone was not enough to detect specific antibodies or for the precise diagnosis of autoimmune disease [17]. Therefore, screening with IIF and specific diagnosis by ANA profile will improve the efficiency of diagnosing patients suspected to have an autoimmune disease. The simultaneous utilization of both methods (i) increases the sensitivity in most cases (ii) provides much more information about the combination of results and (iii) facilitates the interpretation of patterns.

Conclusions

ANA assay with 100% sensitivity and specificity does not exist, clinicians must look to balance sensitivity and specificity. Based on this study, clinicians should test for ANAs only when a CTD is suggested by the patient's history and physical examination findings. It is cost-effective to use a sensitive ANA IFA when screening patient samples. Owing to its low specificity it should be used only for screening new cases, and positive results should be sent for confirmation by ANA profile to determine the presence of specific antibodies.

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