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RESEARCH ARTICLE

Comparative study for detection of antinuclear antibodies by ELISA and Immunoflourescent techniques

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Abstract

Background: Antinuclear antibodies frequently arise in the sera of patients with connective tissue disease, including systemic lupus erythematosus, rheumatoid arthritis. Their serum presence is included in the classification criteria for systemic lupus erythematosus of the American College of Rheumatology. However, antinuclear antibodies can also be found in the absence of autoimmune diseases including various types of infections. **The aim of this study:** was to analyze antinuclear antibody screening by enzyme-linked immunosorbent assay (ELISA) followed by indirect fluorescent antibody assay testing to confirm and characterize and to compare the sensitivities of enzyme-linked immunosorbent assay and indirect fluorescent antibody testing for detection of antinuclear antibody. **Results:** Only one out of the 50 samples from apparently healthy adults had a positive antinuclear antibodies result by the indirect immunofluorescence assay test (99.5% specificity). However, Aesku, and INOVA ELISAs demonstrated specificities of 95%, and 85%, respectively, in the healthy serum samples when compared with indirect immunofluorescence assay results as the standard. Also sensitivities of enzyme-linked immunosorbent assay and indirect fluorescent antibody assay testing using clinically defined serum samples demonstrated that in case of systemic lupus erythematosus patients, Aesku, and INOVA antinuclear antibodies ELISAs demonstrated excellent screening sensitivities of 92.8% and 91.6%, respectively compared with antinuclear antibodies by indirect immunofluorescence assay test which had only 75% sensitivity. The antinuclear antibodies ELISAs, Aesku, and INOVA, detected 45%, and 52% positives, respectively in those clinically diagnosed as rheumatoid arthritis patients compared with less sensitive indirect immunofluorescence assay test , which detected only(30%) positive samples for antinuclear antibodies. **Conclusion:** The results proved that ELISA system is more sensitive for detection of antinuclear antibodies compared with indirect immunofluorescence assay test. **Recommendation:** Our data support the routine use of antinuclear antibodies ELISA screening of patients suspected of having autoimmune diseases of the connective tissue followed by indirect immunofluorescence assays on positive samples for confirmation of antibodies, pattern, and titer.

Introduction

Antinuclear antibodies are antibodies produced by the immune system that are directed against various structures located in the nuclei of the body's own cells. There is a convincing association between these antibodies and autoimmune diseases of the connective tissue. **[1].**

These antibodies probably occur in the circulation of all human beings, but the employed test is only considered 'positive' if they occur at titres elevated significantly above the normal serum level. ANA were first demonstrated in 1957 by Holborow *et al*., using indirect immunofluorescence [2].

Antinuclear antibodies are frequently seen in systemic autoimmune diseases, including systemic lupus erythematosus, scleroderma, polymyositis and dermatomyositis. They also are detected in patients with organ-specific autoimmune diseases, such as autoimmune thyroiditis and hepatitis, certain infections and neoplasms, and in some individuals without diagnosed disease [3 & 4].

Antinuclear antibodies, are traditionally assessed by indirect immunofluorescence and include antibodies to both nuclear and cytoplasmic components [5].

Elevated levels of antinuclear antibodies are found in all systemic rheumatic diseases, with sometimes high, sometimes rather loose associations between a particular antinuclear antibodies specificity and a particular rheumatic disease. Therefore, the detection and identification of antinuclear antibodies has gained increasing acceptance by clinicians who use the information to help or confirm a diagnosis and in treatment follow- up.

After more than 40 yr, indirect immunofluorescence is still used as a screening technique, although the employed substrate has evolved from organ tissue to cultured cells. Since the molecular characterization of most antigens, other techniques, such as enzyme- linked immunosorbent assay (ELISA) and immunoblotting, have been developed that allow the precise identification of many antinuclear antibodies specificities [6].

In 2008, the American College of Rheumatology initiated a task force to investigate and collect information from physicians to evaluate the extent of the problem. In August 2009, the American College of Rheumatology issued a statement declaring indirect immunofluorescence assay as the preferred method for antinuclear antibodies screening [7].

Using HEp-2 cells as the substrate, the indirect immunofluorescence assay allows detection of more than 50 autoantibodies to 30 different nuclear and cytoplasmic antigens. These include antibodies to Golgi apparatus, mitochondria, Jo-1, ribonuclear protein, and others. Although it is ideal to report all fluorescence observed on the HEp-2 cells, many laboratories issue reports based on 5 or 6 basic indirect immunofluorescence assay patterns, namely, homogeneous, speckled, nucleolar, centromere, peripheral/rim, and proliferating cell nuclear antigen, which are titered to end point. Each laboratory independently decides whether to indicate "cytoplasmic fluorescence observed," spindle apparatus (NuMA [nuclear mitotic apparatus]), nuclear dots, or other fluorescent patterns. It is well known that the indirect immunofluorescence assay method is labor-intensive, subjective, and prone to reader bias [8].

The need for standardizing of antinuclear antibodies testing continues to grow, as does controversy about the best test to use [9].

Material

Clinical samples:

A total of one hundred fifty samples were analyzed for this study. The tested samples were grouped as follow:

- Fifty clinically defined systemic lupus erythematosus cases that met American College of Rheumatology criteria[10].
- Fifty clinically defined rheumatoid arthritis cases based on American College of Rheumatology criteria[11].
- Fifty samples from apparently healthy adults (30 males & 15 females).

Kits for detection of ANAs:

- **IFA assay kit** [indirect immunofluorescence assay] (NOVA Lite HEp-2 antinuclear antibodies), (product of INOVA Diagnostics, San Diego, CA), which uses an IgG heavy chain–specific conjugate.
- **ELISA assay kits (enzyme-linked immunosorbent assay)** from two manufacturers:
    a) **Aeskulisa ANA HEp-2** (**HEp-2= human epithelial cell**) (product of Aesku Diagnostics, Wendelsheim, Germany).
    b) **QUANTA Lite ANA ELISA** (product of INOVA Diagnostics, San Diego, CA).

The specific manufacturer's published mix of antigens is shown in Table (1).

Table (1): Antigens Included in the two ELISA kits.

	ELISA Manufacturer	
Antigen	**Aesku**	**INOVA**
HEp-2	**Yes**	**Yes**

Double-stranded DNA	**Yes**	**Yes**
Histone	**Yes**	**Yes**
Smith	**Yes**	**Yes**
Smith/ribonucleoprotein	**Yes**	**Yes**
Ribonucleoprotein	**Yes**	**Yes**
SSA/Ro 60 kDa	**Yes**	**Yes**
SSA/Ro 52 kDa	**Yes**	**Yes**
SSB/La	**Yes**	**Yes**
Scleroderma-70	**Yes**	**Yes**
Centromere B (80 kDa)	**Yes**	**Yes**
Jo-1	**Yes**	**Yes**
Ribosomal protein	**Yes**	**Yes**
Mitochondria 2	**No**	**Yes**

- **ELISA BioTek Instrument** (Model ELx800):(Washer + Microplate Reader +Incubator)NY., USA.
- **Fluorescent microscope** (Model MF220-H LED**)** (product of B W Optics, China).

Methods

All assays using ELISA assay kits; were performed as stated in the manufacturer's product insert. All samples were read on a Fluorescent microscope independently by board certified medical technologist well trained in performing and reading the slides.

Results

Determination of assay specificity in the control serum samples:

Figure 1 shows test characteristics when using two of the ELISAs and the indirect immunofluorescence assay to test fifty apparently healthy adults . Only one of the 50 samples had a positive antinuclear antibodies result by the indirect immunofluorescence assay test. With the indirect immunofluorescence assay test, healthy adult serum samples were reported as less than 1:40 for antinuclear antibodies, demonstrating a negative predictive value of 0.5 or 99.5% specificity.

The Aesku, and INOVA ELISAs demonstrated specificities of 95%, and 85%, respectively, resulting in false positive rates of 5%, and 15%, respectively, in the healthy serum samples when compared with indirect immunofluorescence assay results as the standard.

Sensitivity using clinically defined serum samples

Sensitivity was determined by analyzing the fifty clinically defined systemic lupus erythematosus serum samples that met American College of Rheumatology criteria. As shown in Figure 2, the antinuclear antibodies indirect immunofluorescence assay test had only an 75% sensitivity for the fifty confirmed systemic lupus erythematosus serum samples, while the Aesku, and INOVA antinuclear antibodies ELISAs demonstrated excellent screening sensitivities of 92.8% and 91.6%, respectively.

Figure 3 also shows test characteristics of the two ELISAs and the immunofluorescence assay to test the fifty rheumatoid arthritis serum samples. The antinuclear antibodies ELISAs, Aesku, and INOVA, detected 45%, and 52% positives, respectively, when testing for antinuclear antibodies in the rheumatoid arthritis serum specimens. Only fifteen (30%) of the fifty samples had a positive antinuclear antibodies result by the indirect immunofluorescence assay test. The fifty samples positive by indirect immunofluorescence assay testing were also positive by the two ELISAs.

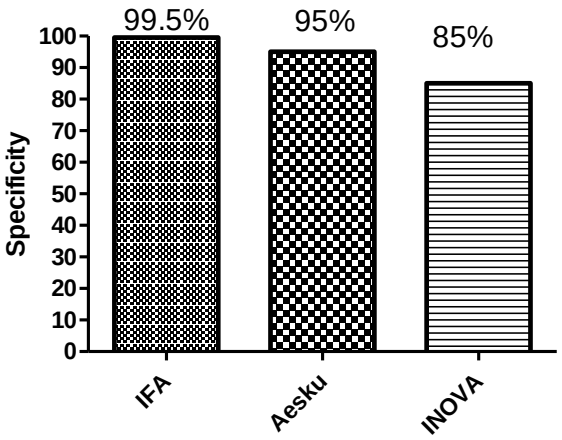

Figure 1: Assay specificities in healthy control serum samples

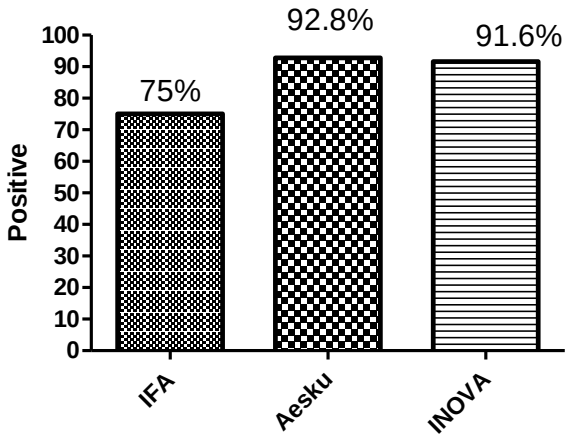

Figure 2: Assay sensitivities in systemic lupus erythmatosus serum samples.

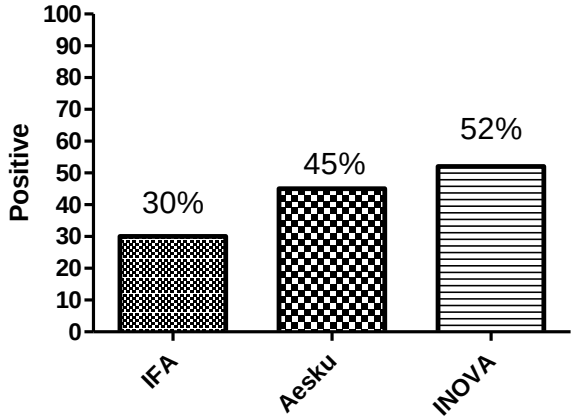

Figure 2: Assay sensitivities in rheumatoid arthritis serum samples.

Discussion

The generation of antinuclear antibodies is an important feature of connective tissue diseases. Several studies have demonstrated that specific antinuclear antibodies are associated with different symptoms of connective tissue diseases**.** Testing for antinuclear antibodies is an initial logical step in evaluating for connective tissue diseases in patients with manifestations suggestive of such a diagnosis. The difficulty in diagnosing connective tissue

diseases has led to an increased number of antinuclear antibodies aasay tests being ordered, often to rule out the possibility of an autoimmune disease [12].

During the past two decades, new methods for detecting and identifying antinuclear antibodies have emerged to address the growing number of antinuclear antibodies tests requested. Along with these newer methods, there has been a growing number of HEp-2 antinuclear antibodies indirect immunofluorescence assays that are commercially available. The growth of antinuclear antibodies testing, new methods, and increased number of manufacturers, unfortunately, have led to a lack of standardization among the assays [13].

The sensitivity, specificity, and predictive value of the test vary owing to the specifics of the assay selected by the laboratory. Antinuclear antibodies indirect immunofluorescence assays testing is also affected by many variables, such as the specificity of the substrate, the conjugate, the microscope bulb, and, especially, the reader.

In this study, we sought to screen for antinuclear antibodies by indirect immunofluorescence assays testing as a routine approach for screening followed by selective use of ELISA and confirming the presence of antinuclear antibodies.

The results of the present study showed higher sensitivities, 92.8% and 91.6%,(for Aesku, and INOVA antinuclear antibodies ELISAs) evaluated compared with 75% sensitivity for the HEp-2 indirect immunofluorescence assays test in the clinically defined systemic lupus erythematosus samples. Also the antinuclear antibodies ELISAs, Aesku, and INOVA, detected 45%, and 52% positives, respectively, when testing for antinuclear antibodies in the rheumatoid arthritis serum specimens. and only fifteen (30%) of the fifty samples of rheumatoid arthritis serum specimens had a positive antinuclear antibodies result by the indirect immunofluorescence assay test. The fifteen samples positive by indirect immunofluorescence assay testing were also positive by the two ELISAs.

The high sensitivity of the ELISA allows it to be used for screening for antinuclear antibodies so that negative serum samples can be reported directly, whereas serum samples positive by ELISA can be confirmed by indirect immunofluorescence assays testing. Using an ELISA with 90% or greater sensitivity would allow a laboratory to report the majority of patient samples, which are negative, at lower cost and with a shorter turnaround time.

Concerning the assay tests pecificities demonstrated in the current study, both indirect immunoflourescence assays and ELISA tests, nearly more or less the same.

The National Committee for Clinical Laboratory Standards (NCCLS) published a guideline for quality assurance of indirect immunofluorescence testing for antinuclear antibodies, which offers a voluntary standard developed by consensus of the clinical laboratory testing community [14].

Several studies reported their comparison of HEp-2 immunofluorescence assays testing with ELISA for antinuclear antibodies testing. They concluded; ELISA prescreening combined with indirect immunofluorescence assays can obtain good information. The combination of two or more testing methods can greatly enhance the accuracy of the results. Also they demonstrated that commercially available antinuclear antibodies ELISAs show different degrees of sensitivity and specificity and that some have a diagnostic accuracy that is comparable to or, in some cases, higher than indirect immunofluorescence assays testing [15 & 16].

Since an antinuclear antibodies 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 antinuclear antibodies only when a connective tissue diseases is suggested by the patient's history and physical examination findings.

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