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Research Article

Sensitivity and Specificity of the Anti-Nuclear Antibody (ANA) Indirect Immunofluorescent Assay (IIFA) at Varying Titers for Diagnosing SLE: An Evidence Based Approach for Assessing the Utility of ANA Tests in the Clinical Setting

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Abstract

Purpose: The anti-nuclear antibody indirect immunofluorescent assay (IIFA ANA) is an important screening tool for rheumatic diseases, particularly lupus. The conventional IIFA ANA positive cutoff titer of 1:40 offers high sensitivity but very low specificity for the clinical diagnosis of lupus. The objective of this study is to correlate in vitro IIFA ANA data with clinical lupus diagnoses on a large scale in order to identify a cutoff titer that maximizes specificity without sacrificing sensitivity of this test.

Methods: A retrospective analysis of 1475 positive IIFA ANA test results from the Cleveland Clinic Foundation was conducted. The medical record of each patient with a positive ANA result was examined to determine if the patient was diagnosed with lupus. The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) for lupus were then calculated for increasing ANA titers: 1:40, 1:80, 1:160,1:320, 1:640, and >1:640.

Results: The sensitivity of the IIFA ANA test drops from 96.2% at a 1:80 titer to 81.0% at a 1:160 titer with only a marginal increase in specificity from 78.0% to 83.8%.

Conclusion: Since the IIFA ANA test is most often used as a screening test for SLE, our data suggest maintaining a lower cutoff titer of 1:40 or 1:80 is still necessary to preserve the high sensitivity of IIFA ANA for lupus screening.

Keywords: Anti-nuclear antibody; Systemic lupus erythematous; Lupus; Sensitivity; Specificity

Introduction

The Anti-Nuclear Antibody (ANA) indirect immunofluorescent assay (IIFA) is the gold-standard screening test for Systemic Lupus Erythematosus (SLE) and lupus subtypes. However, there are few studies correlating IIFA ANA test results at different titers with the clinical diagnosis of lupus. Additionally, studies have found a high fraction of false positive results at the conventional cutoff titer of 1:40 [1-3]. In theory, a cutoff titer that is too low leads to unnecessary referrals to rheumatologists and exposes patients to avoidable drug exposure and side effects. It has been postulated that a higher IIFA ANA cutoff titer could offer better specificity for the detection of SLE; however, it is unclear if this would lead to a significant loss of sensitivity. To test this hypothesis, a retrospective analysis was conducted to determine the sensitivity, specificity, positive predictive value, and negative predictive value of the IIFA ANA test for SLE and lupus subtype screening at various titers in a well-characterized clinical population.

Method

The IIFA ANA tests used in this study were interpreted in a standardized manner by experienced clinical pathologists at the Clinical Immunology Reference Laboratory - Cleveland Clinic Foundation. Briefly, HEp 2 cells (Fluorescent HEp-2, Immuno Concepts, and Sacramento, CA) were incubated with patient serum at 1:40 dilution. After washing, slides were incubated with anti-immunoglobulin antibodies bound to FITC – fluorescein isothiocynate. After washing and cover-slipping, the slides were examined under a fluorescent microscope by a trained medical technologist. Bright apple-green fluorescence is seen in a characteristic pattern in positive cases. All positive cases were diluted to an end dilution up to 1:640. Samples with positive staining at 1:640 were resulted as >1:640. A total of 9361 IIFA ANA test results conducted between January 1, 2013 and November 1, 2013 were made available for this study using the laboratory information system (Sunquest, Tucson, AZ). 72 samples were removed for either incomplete information or redundancy

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Table 1: Raw data from patients who underwent lupus screening with the IIFA ANA test at various titers.

ANA IIFA	Test result	Total	True Positive	False Positive	True Negative	False Negative
1:40	Positive	1475	158	1317	3838	0
	Negative	3838				
1:80	Positive	1285	152	1133	4022	6
	Negative	4028				
1:160	Positive	963	128	835	4320	30
	Negative	4350				
1:320	Positive	716	103	613	4542	55
	Negative	4597				
1:640	Positive	452	68	384	4771	90
	Negative	4861				

within the data set. 5313 of the remaining samples had accessible clinical data through the medical record (EPIC, Kansas City, MI) system. 1475 of these samples were ANA positive, defined by a 1:40 titer or greater and 3838 were ANA negative. The medical record of each patient with a positive ANA result was examined to determine if the patient met ACR clinical criteria for the diagnosis of SLE or a lupus subtype. A systematic approach was taken to determine if a patient had a true diagnosis of lupus. First, the assigned problem list for a patient within the medical record was explored. If a lupus diagnosis was identified then the clinical note specifying the diagnosis was located to confirm that an appropriate clinical workup was done. In all positive cases, a clinical workup at The Cleveland Clinic Foundation, an affiliated hospital, or an outside hospital supporting the lupus diagnosis was identified. Cases in which a medical history was positive for lupus with no clinical workup documentation were not called positive. In positive cases where a patient was tested multiple times with discordant titer results, the lowest positive titer was selected as the data point. Prior studies report high sensitivity of IIFA ANA for the clinical diagnosis of lupus so it was assumed that all ANA negative samples were clinically negative for lupus. The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) for lupus were then calculated for each ANA titer: 1:40, 1:80, 1:160, 1:320, 1:640, and >1:640 using excel (Microsoft corp. Seattle, WA.) software. The following are the formulae used for calculating pertinent data:

Sensitivity = TP/TP + FP

Specificity = TN/FP+ TN

NPV = TN/ (FN + TN)

PPV = TP/ (TP + FP)

Where TP= true positive, FP= false positive, TN= true negative, FN= false negative,

PPV= positive predictive value and NPV= negative predictive value.

Results

Of the 1475 positive IIFA ANA test results 1189 were from female patients and 286 were male with an overall average age of 51. There were 167 cases of lupus with documented supporting clinical evaluation. The vast majority of these cases were SLE. There were four cases of discoid lupus and three cases of drug-induced lupus that were included as lupus positive cases in this study. One case of discoid lupus was ANA positive at a titer of 1:80, two were 1:160, and one was 1:320. All three cases of drug-induced lupus were ANA positive at a titer of 1:320. The raw data correlating IIFA ANA titer and clinical lupus diagnosis is shown in (Table 1). The sensitivity, specificity, positive predictive value, and negative predictive value of each IIFA ANA titer for the clinical diagnosis of lupus is shown in (Table 2).

Discussion

As expected, a decreasing sensitivity and increasing specificity is noted as the IIFA ANA titer increases. The statistical metrics calculated in this study suggest similar test performance for the 1:40 and 1:80 titers with a significant decrease in sensitivity observed at a titer of 1:160. These data are in contrast to a recent smaller study suggesting little change in IIFA sensitivity for the detection of rheumatic diseases as a whole up to a titer of 1:320 [1]. Recent international guidelines suggest a 1:160 IIFA ANA titer is often suitable for rheumatic disease screening in adults; however, an emphasis is also placed on locally defining this titer based on internally optimized standards to exclude 95% of healthy controls [4]. One limitation of our retrospective large-scale study is the inability to stratify data in between the predetermined

Table 2: Sensitivity, Specificity, PPV, and NPV data for each of the ANA titer groups.

1:40	Sensitivity	100.0%*
	Specificity	74.5%
	PPV	10.7%
	NPV	100.0%*
1:80	Sensitivity	96.2%
	Specificity	78.0%
	PPV	11.8%
	NPV	99.9%
1:160	Sensitivity	81.0%
	Specificity	83.8%
	PPV	13.3%
	NPV	99.3%
1:320	Sensitivity	65.2%
	Specificity	88.1%
	PPV	14.4%
	NPV	98.8%
1:640	Sensitivity	43.0%
	Specificity	92.6%
	PPV	15.0%
	NPV	98.1%
1:>640	Sensitivity	24.7%
	Specificity	95.8%
	PPV	15.2%
	NPV	97.6%

*The sensitivity of the IIFA ANA test at the 1:40 titer was not evaluated in this study and is assumed to be 100% based on prior studies.

dilutions, which made it difficult to scrutinize the drop-off in test sensitivity between 1:80 and 1:160. Even small changes in IIFA ANA dilution can impact data substantially as demonstrated in a 2008 study with 300 healthy blood donors that found the rate of IIFA ANA false positivity dropped substantially from a 1:40 titer to 1:50 titer [5]. Another important limitation of this study is that samples that tested negative by IIFA ANA were not evaluated for a clinical diagnosis of lupus. Rather, it was assumed that the test was sufficiently sensitive at the 1:40 dilution based on prior studies suggesting >95% sensitivity, particularly since the use of HEp-2 cells has become an industry standard [6]. However, it has also been demonstrated that ANA-negative lupus can and does occur, particularly in patients with photosensitivity and SSA/Ro antibody positivity [7] and possibly in cases with established SLE [5].

Conclusion

Our data suggest a lower screening titer of 1:40 or 1:80 is necessary in this clinical setting to maintain the high sensitivity of this test for lupus screening. Follow-up in vitro testing such as anti-double stranded DNA, complement levels, and clinical evaluation remain the mainstay of improving specificity for the suspicion of rheumatic disease diagnosis. Nonetheless, clinical judgment in restricting ANA screening to individuals with a higher pre-test probability of lupus or other ANA-positive rheumatic disease can help limit the occurrence of false positive IIFA ANA results.

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