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# Harmonization of clinical interpretation of antinuclear antibody test results by solid phase assay and by indirect immunofluorescence through likelihood ratios

Antinuclear antibodies (ANAs) are helpful in the clinical diagnosis and classification of ANA-associated rheumatic diseases (AARDs), such as systemic lupus erythematosus (SLE), systemic sclerosis (SSc), primary Sjögren's syndrome (SjS), idiopathic inflammatory myopathies (IIM) and mixed connective tissue disease (MCTD) [1].

ANAs have traditionally been detected by indirect immunofluorescence (IIF) on HEp-2 cells, but alternative fully automated solid-phase immuno-assays such as chemiluminescence immunoassay (CIA) and fluoroenzyme (FEIA) immunoassay are increasingly used. Such automated immunoassays measure specific antibodies to a set of selected autoantigens associated with AARDs.

Generally, IIF is considered highly sensitive (but with low specificity), whereas FEIA and CIA are considered to be more specific (but with lower sensitivity). In a recent evaluation, it was reported that the area under the curve (AUC) of the receiver operating characteristics (ROC) curve was comparable between CIA, FEIA and automated IIF [2]. This suggested that the overall performance of the three assays was comparable and that differences in sensitivity and specificity are related to the choice of the cutoff values. Just recently, a meta-analysis was performed comparing IIF and solid phase assays which concluded that both methods provide comparable performance characteristics [3]. Standardizing the numerical values reported in results obtained by IIF, FEIA and CIA is unrealistic given the differences in methodology. However, harmonizing clinical interpretation of the different ANA/AARD screening assays might be achievable (to a certain level) by providing test result-specific likelihood ratios (LRs).

We estimated test result-specific LRs for AARD for IIF, CIA and FEIA in a group of patients and controls that have been previously described [2]. The analyses were performed on (i) 480 diagnostic samples from AARD patients [SLE (n = 119), primary SjS (n = 65), SSc (n = 220), IIM (n = 50), MCTD (n = 56)] and (ii) samples from 767 controls [diseased controls (n = 314), chronic fatigue syndrome (n = 150) and blood donors (n = 279)]. All AARD patients fulfilled classification criteria [2].

ANA was performed by automated IIF (NOVA View, Inova Diagnostics, San Diego, CA, USA), CLIA (QUANTA Flash CTD Screen Plus, Inova Diagnostics) and FEIA (EliA CTD screen, Thermo Fisher, Freiburg, Germany). NOVA View screens for ANA at a serum dilution of 1/80 [4]. For CIA, the antigens are dsDNA, Sm/RNP, Ro60/SSA, Ro52/TRIM21, SS-B/La, centromere (A & B), Scl-70/topoisomerase I, Mi-2, RNA-Pol III, PM/Scl, Jo-1, PCNA, Rib-P protein (C22 peptide), Ku, and Th/To. For FEIA, the antigens are ds-DNA, Sm, SSA/Ro60 and Ro52/TRIM21, SS-B/La, Centromere B, Scl-70/ topoisomerase I, Mi-2, RNA-Pol III, PM/Scl, Jo-1, PCNA, Rib-P protein, U1-RNP (RNP-70, A, C), and fibrillarin.

The LRs were calculated using three approaches. The first approach was the 'finite difference' approach in which the derivative of the ROC curve is calculated by locally estimating its derivative by a finite difference formula in each interval i [$LR_i = (y_{i+1}\text{-}y_i)/(x_{i+1}\text{-}x_i)$]. The second approach calculated the slope of the tangent to the ROC curve by adjusting Bézier curves to the ROC that are defined by tangents to the curve [5]. A simplified method to adjust (shape control) a cubic Bézier curve to a ROC curve was used [6] [7] (Supplemental Data). The third approach estimated the distribution of the diseased and control population using a sliding interval over the measured values [$\log(x_i)$, $t^*\log(x_i)$]. The LRs are calculated by dividing the frequency of diseased cases over the frequency of controls in each interval.

Fig. 1 shows the distribution of the results for AARD and controls as well as the estimations of the LRs for FEIA, CIA and automated HEp-2 IIF. The maximal Youden index (value for which the sum of sensitivity and specificity is maximal) is shown as well.

For all three methods, test result-specific LRs increased with increasing antibody level. Table 1 gives an overview of the test results that corresponded to a LR of 0.1, 0.33, 1, 3 and 10. A LR of 10 (0.1) indicates that the chance to find such result is 10 times higher (lower) in patients than in controls. Thus, results that are above the 10 LR threshold are useful to aid in the diagnosis of a disease, whereas results below the 0.1 LR threshold are useful to aid in the exclusion of a disease.

The thresholds that corresponded to the maximal Youden index (sensitivity + specificity - 1) were ratio 1, 28.5 CU and 305 LIU, for FEIA, CIA and automated IIF, respectively, which were close to the thresholds that correspond to a LR of 1. When the manufacturer's cutoff are applied for the automated IIF, FEIA and CIA, LRs of 0.1, 1.5 and 0.5, respectively, were observed, which relate to a higher sensitivity for IIF and a higher specificity for FEIA.

Current immunoassays for ANA detection typically rely on a single cutoff point (positive/negative). Using a single cutoff (positive/negative), however, ignores the clinical value contained in the antibody levels (the higher the antibody level, the higher the likelihood of disease). Test result-specific LRs convey important clinical information inherent in the antibody levels. LRs provide an estimation of the clinical significance of a test result: the post-test odds for disease can be estimated by multiplying the pre-test odds by the LR.

A debate that started more than a decade ago focused on the variability in ANA testing, the value of IIF vs. solid phase immunoassays, and the interpretation/harmonization of ANA testing [8–17]. Efforts are needed to improve and align clinical interpretation.

We show that by defining test result-specific LRs, clinical interpretation of ANA test results can be improved and harmonized across

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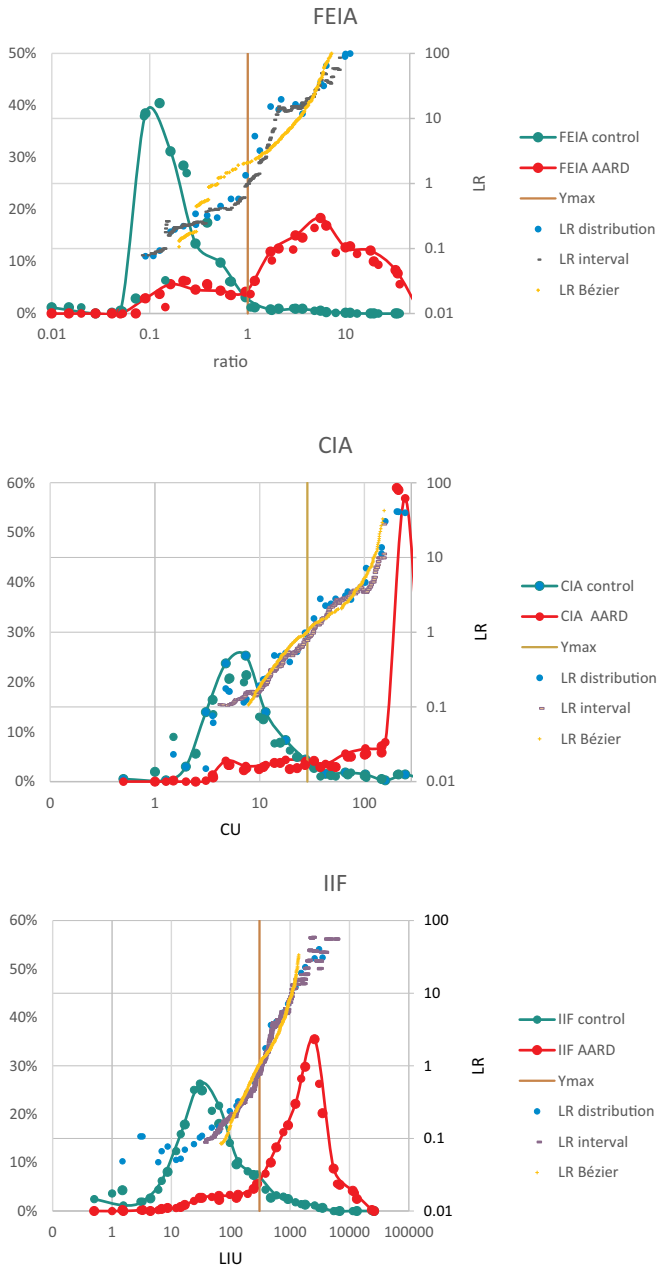


**Fig. 1.** Distribution of test results and test result-specific likelihood ratios (LR).
Patients with AARD (n = 480) and controls (n = 767) were tested for ANA by IIF (NOVA), by CIA (INOVA) and by FEIA (Elia).
The distribution for controls (green line and filled circles) and AARD patients (red line and filled circles) is given on the left Y-axis. The LRs are given on the right Y-axis. Three different ways to calculate the test-result specific LR were applied: (i) the finite difference approach (LR interval), (ii) an approach based on the distribution of the controls and patients (LR distribution) and (iii) the Bézier approach (LR Bézier). The test result that corresponds to the maximal Youden index (sensitivity + specificity - 1) (Y max) is indicated by a vertical line.

assays and suppliers. For this study, all AARDs were grouped. It should be noted, however, that at the level of the individual diseases, there might be differences between immunoassays. For example, for SjS the AUC was higher for CIA and FEIA than for IIF, whereas for SSc the AUC was higher for IIF than for CIA [2]. Thus, while the ANA IIF is considered useful in screening for SLE, SSc and MCTD, it is less sensitive in screening for SjS and an ever-widening spectrum of IIMs [16].

Assigning LRs to a specific test result is a novel approach in the field of ANA testing that can improve interpretation of test results and comparability of different test methods. Such an approach, however, needs well designed studies and collaboration between diagnostic companies, laboratory professionals and clinicians.

## Declaration of Competing Interest

XB has been a consultant for Inova Diagnostics and Thermo Fisher. MJF has been a consultant for Inova Diagnostics. MM is employed by Inova Diagnostics.

Inova Diagnostics provided reagents to perform the study.

**Table 1**
Assay-specific (IIF, CIA, FEIA) test results corresponding to a likelihood ratio of 0.1, 0.33, 1, 3 and 10.

| | IIF NOVAView (LIU) | CIA CTD Screen Plus (CU) | FEIA EliA CTD Screen (ratio) |
|---|---|---|---|
| LR 0.1 | 46/30/70 | 4.5/7/8 | 0.12/0.14/0.21 |
| LR 0.33 | 143 /133/143 | 13/13/13 | 0.30/0.29/0.28 |
| LR 1 | 306/307/302 | 27/27/28 | 0.7/0.9/0.5 |
| LR 3 | 452/445/627 | 70/66/75 | 1.4/1/1 |
| LR 10 | 990/1000/1068 | 133/145/125 | 2.7/3/3.5 |
| Company cutoff | 48 | 20 | 1 |

Three values are given; the first value is obtained from applying the finite difference approach to calculate the LR, the second value from the distribution approach and the third value from the Bézier approach.
The cutoff values proposed by the company are indicated as well.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.autrev.2019.102386.

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