Comparison of the Clinical Utility in the Detection of Anti-Nuclear Antibodies Between the Elia CTD Screen and Indirect Immunofluorescence on Hep-2 Cells: A Review of the Literature

Christoph Robier MD[1,4] Omid Amouzadeh-Ghadikolai MD[3], Mariana Stettin MD[1] and Gerhard Reicht MD[2]

[1]Institute of Laboratory Diagnostics and Departments of [2]Internal Medicine and [3]Psychiatry, Hospital of the Brothers of St. John of God, Graz, Austria
[4]Clinical Institute of Medical and Chemical Laboratory Diagnostics, Medical University of Graz, Austria

The detection of antinuclear antibodies (ANA) is important in the diagnosis of systemic autoimmune disorders such as systemic lupus erythematosus (SLE), systemic sclerosis (SSc), Sjögren's syndrome, mixed connective tissue disease (MCTD), and polymyositis. ANA are thus incorporated in several classification criteria [1,2]. Beyond that, there is an open discussion whether ANA represent useful biomarkers in preventive medicine [3], especially for the identification of pre- or early disease with an "intent to prevent" approach [2,4].

Indirect immunofluorescence (IIF) on Hep-2 cells is regarded as the gold standard for ANA screening and has been defined as the reference screening method for ANA in the clinical laboratory routine [1]. However, it has been shown that some subtypes of ANA, especially anti-SSA/Ro and anti-Jo-1 antibodies, may be overlooked by IIF [5]. Furthermore, IIF requires experienced and well-trained analysts, is quite time-consuming, and shows high inter-observer variability [6]. To overcome these disadvantages, commercially available automated solid-phase assays incorporating a mixture of various extracted antigens have been developed. For laboratories with a high throughput of samples, such automated tests may be beneficial. The diagnostic potential of automated assays is restricted to the antigens incorporated in the panel of the test, which is a clear limitation for the use of solid-phase assays. Furthermore, it has been reported that some clinically relevant ANA included in the panel of these tests may be missed [7].

The use of assays other than IIF is in accordance with international recommendations. However, considering that if the

> **Immunofluorescence on Hep-2 cells and Elia connective tissue disease screening assay differ significantly in the sensitivity and specificity for antinuclear antibodies-associated disorders**

clinical suspicion is strong and the alternative method is negative, it is mandatory to perform IIF [1]. Because both IIF and solid-phase assays have positive properties, in addition to weak points, a combination of the two techniques may improve the screening procedure for ANA.

In this review, we discuss studies that have evaluated the Elia CTD Screen (ECS) assay (Phadia, Thermo Fisher Scientific, Freiburg, Germany) to determine the optimal role of this test in the diagnostic process.

ECS ASSAY

The ECS is a solid-phase fluoroenzymeimmunoassay. Each well of the ECS is coated with the following 17 antigens: dsDNA, SSA/Ro 52, SSA/Ro 60, SSB/La, U1-RNP (RNP70, A, C), Sm, CENP-B, Jo-1, Scl-70, Rib-P, fibrillarin, RNA polymerase III, PM-Scl, PCNA, and Mi-2. All of the antigens that are included, except for dsDNA which is a native purified antigen, are human recombinant proteins. The cut-off levels for all antibody results, with the exception of U1RNP, are: negative < 7 U/ml, equivocal 7–10 U/ml, and positive > 10 U/ml. Cut-off for U1RNP antibody results are: negative < 5 U/ml, equivocal 5–10 U/ml, equivocal, and positive > 10 U/ml [8].

CONCORDANCE OF THE ECS WITH IIF

In some published studies, the concordance between the ECS and IIF has been determined. The concordance is calculated by the sum of all ECS[positive]/IIF[positive] and ECS[negative]/IIF[negative] study subjects. The available studies showed similar results, reporting concordance rates of 78.8% [9], 79.2% [10], and 83.1% [8], respectively.

DIFFERENCES IN THE DETECTION OF ANA BETWEEN THE ECS AND IIF

IIF and automated solid-phase assays have totally different test characteristics. IIF enables the analyst to detect a broad spectrum of antibodies at one view, whereas automated immunoas-

says are limited to the panel of antigens incorporated in the test system. Therefore, the two techniques show differences in the detection of ANA.

In our study, we observed ECS^{positive}/IIF^{negative} results in 2.2% and ECS^{negative}/IIF^{positive} results in 16.9% of 1708 subjects. The ECS^{positive}/IIF^{negative} specimens (except from dsDNA-, Ro/SSA-, and Jo-1 antibodies) mostly contained low antibody concentrations of histone, SSB/La, Sm, Scl-70, and U1-RNP. In the ECS^{negative}/IIF^{positive} group, except from one centromere-B antibody in a patient with limited scleroderma, only antibodies not included in the ECS panel (e.g., histone, nucleosome, Pl-12 and AMA-M2) were detected [9]. In a large Italian study population, ECS^{positive}/IIF^{negative} results occurred in 2% (mainly Ro52, Ro60, dsDNA, PM/Scl, and Jo-1), and ECS^{negative}/IIF^{positive} results were found in 31% [11]. These findings are in accordance with previously published studies that revealed that ECS is able to detect antibodies that are missed by IIF, and vice versa, IIF may detect relevant antibodies that are missed by ECS [5,8,12,13].

There is another point that merits mention. It has been shown that even antibodies that are included in the panel of automated assays may be missed. For example, Parker and colleagues [7] observed that the ECS is a sensitive method for the detection of anti-PM-Scl, anti-Mi-2, anti-PCNA, and anti-Rib-P antibodies, but is a suboptimal screening tool for anti-fibrillarin and anti-RNA polymerase III antibodies, with a detection rate of 68% and 67%, respectively.

DIFFERENCES BETWEEN ECS AND IIF IN THE SENSITIVITY FOR ANA-ASSOCIATED SYSTEMIC AUTOIMMUNE DISORDERS

Based on the different diagnostic sensitivity for various ANA subtypes, several studies reported that ESC and IIF also show a different diagnostic performance for ANA-associated autoimmune disorders.

In one study, the sensitivity was higher for IIF than ESC for SLE and SSc, but not for Sjögren's syndrome. A higher specificity was observed for the ESC [14].

In a study conducted by our group, ECS had a 100% sensitivity for Sjögren's syndrome, SSc, and MCTD. The sensitivity for Sjögren's syndrome was higher for ESC than for IIF (94%). IIF had a higher diagnostic sensitivity for SLE, indetermined connective tissue disease, Raynaud's syndrome, and limited scleroderma, compared to the ESC (100% vs. 80%; 100% vs. 75%; 89% vs 57%; 100% vs. 88.9%, respectively) [9].

The authors of a recently published study reported an overall positivity rate for ANA-associated autoimmune disorders of 90% for IIF and 92% for the ESC. In that study, the positivity rate

Combined screening with immunofluorescence on Hep-2 cells and Elia connective tissue disease screen assay enhances the diagnostic accuracy for antinuclear antibodies screening

Antinuclear antibodies (ANA) screening solely by Elia connective tissue disease screening assay might be justifiable in a setting with a low pretest probability for ANA-associated disorders

between IIF and ESC for SLE, Sjögren's syndrome, SSc, MCTD, and PM/dermatomyositis was 93% vs. 98%, 75% vs. 81%, 100% vs. 50%, 100% vs. 100%, and 100% vs. 100%, respectively. Furthermore, IIF showed a positive result in all patients with an ANA-associated disorder in remission, whereas ESC was positive in only 22% [10]. In a trial comparing two solid-phase assays with IIF, the area under the receiver operating characteristics curve (AUC) for Sjögren's syndrome was higher for the solid-phase assays than for IIF; whereas for SSc, the AUC was higher for IIF than for a solid-phase test [15]. Finally, in a recently published paper by Willems and co-authors [8], IIF and ESC were positive in 90.4% and 69.9% of SLE, 100% and 84.1% of SSc, 86.7% and 93.3% of Sjögren's syndrome, 88.2% and 52.9% of PM/dermatomyositis, and in all cases of MCTD.

WHAT SHOULD BE THE PREFERRED SCREENING STRATEGY FOR ANA?

Since solid-phase assays and IIF differ significantly in the sensitivity and specificity for various ANA-associated autoimmune disorders [15], it seems likely to establish a combined screening algorithm incorporating both techniques, either in a sequential or a parallel screening approach. Bossuyt and Fieuws [14] recommended that favorable strategies should be disease-dependent. In their study, the best diagnostic strategy for SLE and Sjögren's syndrome was to perform both tests on all samples; whereas for SSc, screening with IIF and performing a solid-phase assay on IIF-positive samples was comparable to both IIF and solid-phase assay on all samples. Bizzaro et al. [11] showed that the association of solid-phase screen assays to IIF increases the sensitivity from 89.2% to 97.4% and the specificity from 64.6% to 98.4% in serological tests for ANA screening. In addition, an analysis of costs demonstrated that the combination of the two techniques represents a cost-effective diagnostic pathway, reducing the global costs for the immunoserological diagnosis of ANA-associated autoimmune disorders by 22% [11].

CONCLUSIONS

Several studies have confirmed that ESC and IIF differ in the diagnostic sensitivity for the various types of ANA-associated systemic autoimmune diseases. Combined screening with IIF and ESC enhances the diagnostic sensitivity and specificity for ANA, and beyond that, markedly reduces the costs for the laboratory diagnosis for ANA-associated disorders. Screening for ANA solely by ECS might be justifiable in a clinical setting with a low pretest probability for ANA-associated systemic autoimmune disorders. However, in cases of a clinical suspicion of ANA-associated disease and a negative ECS, additional IIF should be performed [1,8,10].

Correspondence
Dr. C. Robier
Institute of Laboratory Diagnostics, Hospital of the Brothers of St. John of
God, A-8020 Graz, Austria
Phone: (43-316) 5989-26671, **Fax:** (43-316) 5989-21505
email: christoph.robier@bbgraz.at

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Capsule

Stitching peptides for presentation

Intracellular protein-derived peptides generated by proteasomal degradation are loaded onto major histocompatibility complex (MHC) class I molecules in the endoplasmic reticulum and presented to CD8+ T cells. Although it has been assumed that these peptides are contiguous segments derived from intracellular proteins, recent studies have shown that noncontiguous peptides generated by cis-splicing of two distinct regions of an antigen can be presented by MHC class I molecules. **Faridi** and colleagues demonstrated that MHC class I molecules can present peptides that are generated by the splicing together of segments from two distinct proteins, so-called trans-spliced peptides. Precisely how cis- and trans-spliced peptides are generated and how they contribute to T cell selection and expansion remain to be explored.

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Eitan Israeli

Capsule

Pathogen elimination by probiotic *Bacillus* via signaling interference

Probiotic nutrition is frequently claimed to improve human health. In particular, live probiotic bacteria obtained with food are thought to reduce intestinal colonization by pathogens, and thus to reduce susceptibility to infection. However, the mechanisms that underlie these effects remain poorly understood. **Piewngam** and colleagues reported that the consumption of probiotic *Bacillus* bacteria comprehensively abolished colonization by the dangerous pathogen *Staphylococcus aureus* in a rural Thai population. The authors showed that a widespread class of *Bacillus* lipopeptides, the fengycins, eliminates *S. aureus* by inhibiting *S. aureus* quorum sensing, which is a process through which bacteria respond to their population density by altering gene regulation. This study presents a detailed molecular mechanism that underlines the importance of probiotic nutrition in reducing infectious disease. The authors also provided evidence that supports the biological significance of probiotic bacterial interference in humans, and shows that such interference can be achieved by blocking a pathogen's signaling system. Furthermore, these findings suggest a probiotic-based method for *S. aureus* decolonization and new ways to fight *S. aureus* infections.

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Eitan Israeli