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Optimizing the Diagnostic Algorithm for Systemic Autoimmune Diseases: Navigating ANA/anti-ENA Discordance

Touimi Benjelloun Hatim[1], Fahi Mohammed[1,2], Meftah Ahlam[1,2], El Bakkouri Jalila[1,2]

[1]Immunology Laboratory, Ibn Rochd University Hospital, Casablanca, Morocco

[2]Clinical Immunology and Immuno-Allergy Laboratory (LICIA), Hassan II University, Faculty of Medicine and Pharmacy, Casablanca

ABSTRACT

Background: The diagnostic workup for systemic autoimmune diseases typically follows a sequential algorithm, beginning with antinuclear antibody (ANA) screening via indirect immunofluorescence (IIF) followed by specific anti-extractable nuclear antigen (anti-ENA) testing. Despite its robustness, clinicians frequently encounter biological discrepancies—such as ANA-positive/anti-ENA-negative or ANA-negative/anti-ENA-positive results—that can lead to diagnostic uncertainty, unnecessary medical costs, or delayed treatment.

Objectives: This review aims to analyze the technical and biological mechanisms underlying these discrepancies and proposes an optimized, pragmatic decision-making framework based on clinical probability.

Methods: We examine the foundations of the traditional diagnostic algorithm, focusing on the sensitivity and specificity of IIF on HEp-2 cells compared to targeted ENA panels. Technical factors such as antigen solubility ("wash-out" effect), substrate variation, and the clinical significance of specific patterns (e.g., AC-2/anti-DFS70) are evaluated.

Results: ANA-positive/anti-ENA-negative results often stem from low-titer non-specific positivity, targets not included in standard panels, or pre-clinical phases. Conversely, ANA-negative/anti-ENA-positive cases, though rarer, are clinically critical and often involve specific antibodies like anti-SSA/Ro, which may be undetectable by standard IIF due to antigen accessibility or technical interference.

Conclusion: Effective diagnosis requires moving beyond strict algorithmic adherence toward a "nuanced interpretation" model. An optimized approach integrates IIF titers and staining patterns with pre-test clinical probability. Ultimately, clinical suspicion must take precedence over discordant laboratory results to prevent under-diagnosis in symptomatic patients and over-diagnosis in asymptomatic individuals.

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KEYWORDS:

Antinuclear Antibodies (ANA), Extractable Nuclear Antigens (anti-ENA), Indirect Immunofluorescence (IIF), Diagnostic Discrepancy, Systemic Connective Tissue Diseases (CTD), HEp-2 Cell Patterns (ICAP)

INTRODUCTION

Interpreting antinuclear antibodies (ANA) remains a frequent source of clinical uncertainty. While widely prescribed as a sensitive screening tool, their low specificity (1) often leads to over interpretation when disconnected from a suggestive clinical context.

Corresponding Author: Fartmissi Manar

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The traditional sequential strategy—starting with ANA followed by anti-ENA—relies on indirect immunofluorescence (IIF) as a sensitive filter to rule out systemic connective tissue diseases before defining the phenotype through specific testing (1). However, clinicians often face discrepancies: ANA positivity without anti-ENA, or isolated anti-ENA with a negative ANA. These scenarios create genuine diagnostic pitfalls, resulting in either exhaustive, unnecessary workups or delayed management.

This review analyzes the underlying mechanisms of these discrepancies and proposes a pragmatic decision-making algorithm based on pre-test clinical probability.

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FOUNDATIONS OF THE DIAGNOSTIC ALGORITHM

Biological Rationale

The ANA-to-anti-ENA algorithm is built on the successive leverage of test sensitivity and specificity. Indirect immunofluorescence (IIF) using HEp-2 cells (2) detects a broad spectrum of nuclear and cytoplasmic antibodies. Its high sensitivity makes it the ideal screening tool to rule out systemic connective tissue diseases. However, its low specificity carries a risk of overdiagnosis, as low titers are frequently observed in healthy individuals (3) depending on the clinical context. In contrast, anti-ENA antibodies (SSA, Sm, RNP, Scl-70, Jo-1) target specific extractable nuclear antigens and are more strictly associated with defined clinical entities. These markers allow for a more refined diagnosis, phenotyping, and prognostic assessment.

Clinical and Economic Rationale

A sequential approach avoids redundant testing. Routinely ordering a full anti-ENA panel as a first-line measure incurs unnecessary costs and increases the likelihood of finding clinically irrelevant, incidental results. Therefore, ANA testing acts as a biological filter, limiting specific investigations to cases where they are truly justified. When applied to patients with a genuine clinical suspicion, this strategy effectively rules out the suspected disease or narrows down investigations, thereby reducing medical waste (4).

Discrepancy Scenarios: Understanding the Mechanisms

While the sequential approach is robust, clinical practice frequently reveals biological divergences. These discrepancies should not be viewed as errors; rather, they reflect the inherent technical limitations of immunofluorescence and the complex nature of autoantibodies.

Mechanisms of ANA-Positive / Anti-ENA-Negative Discrepancies

This is the most common clinical scenario and is driven by several factors:

- **Low-Titer Non-Specific Positivity:** Frequently observed in healthy or elderly individuals, these titers often rise during transient inflammation, pregnancy, or as a result of specific drug therapies (5).
- **Targets beyond Standard ENA Panels:** IIF can detect antibodies that are simply not included in conventional commercial kits. This includes rare specificities in systemic connective tissue diseases (e.g., anti-Ku) or antibodies associated with organ-specific conditions, such as autoimmune liver disease or thyroiditis.
- **Anti-DFS70 Antibodies:** These are relatively common in healthy subjects and can account for isolated ANA positivity without any underlying pathological significance (6).
- **Pre-clinical Phase:** ANA positivity may precede the development of symptoms and the appearance of anti-ENA by several years (7).

Clinical Implication: An isolated positive ANA, particularly at a low titer and in the absence of suggestive clinical features, should not trigger systematic diagnostic escalation.

Mechanisms of ANA-Negative / Anti-ENA-Positive Discrepancies

Although rarer, this configuration is clinically critical and can be explained by:

- **IIF Sensitivity and Antigen Accessibility:** Certain antibodies, such as anti-SSA/Ro, may be present at concentrations undetectable by standard fluorescence (8). Furthermore, the cytoplasmic distribution or low expression of some antigens on HEp-2 cells can limit their visualization.
- **Technical Limitations:** Variations in substrates, prozone effects, or analytical interferences can sometimes mask the reaction.
- **Antigen Solubility and Expression:** Soluble antigens (notably Ro/SSA) may be "washed away" during the IIF fixation process, rendering the staining undetectable despite the presence of antibodies (9). Additionally, insufficient expression of Ro/SSA in certain HEp-2 cell lines can lead to false-negative ANA results.

Clinical Implication: A negative ANA result does not rule out systemic connective tissue disease if clinical suspicion remains high (e.g., sicca syndrome, myositis, or subacute lupus). In such cases, testing for anti-ENA should take precedence over the strict algorithmic protocol.

Optimizing Testing Strategies: Standard vs. Targeted Panels

When systemic connective tissue disease (CTD) is suspected, the choice of second-line testing must be strictly guided by the specific clinical presentation.

- **Standard ENA Panels:** These are indicated for the initial screening of undifferentiated CTDs. While they cover the most common targets (Sm, RNP, SSA, SSB, Scl-70, Jo-1), their diagnostic yield may be insufficient for identifying more specific or rare pathologies.
- **Targeted Panels and Specific Assays:** These should be prioritized whenever a distinct clinical entity is suspected. This is particularly crucial in cases of negative ANA results or when exploring complex phenotypes such as inflammatory myopathies or systemic sclerosis.
- **The Case of Anti-DFS70:** Testing for anti-DFS70 may be considered when ANA testing reveals a dense fine speckled pattern (AC-2). When found in isolation, these antibodies are frequently observed in

individuals without any underlying systemic CTD (10).

The following table summarizes the correlations between clinical suspicion and the specific antibodies to be sought when the clinical scenario warrants a departure from the standard panel:

Table 1: Clinico-biological correlations and targeted testing strategies

Clinical Suspicion	IIF Pattern (ICAP)	Recommended Testing	Key Antigens	Rationale for Targeted Testing
Sjögren's Syndrome	Fine speckled (AC-4) or Negative	Isolated anti-SSA and anti-SSB	Ro/SSA (60 and 52 kDa)	Risk of SSA "wash-out" during IIF (Negative ANA).
Myositis / Myopathies	Cytoplasmic (AC-19, 20) or Fine speckled	Myositis Panel (Dot/Blot)	Jo-1, PL-7, PL-12, Mi-2, SRP	Antigens are often cytoplasmic or poorly visualized on IIF.
Systemic Sclerosis	Nucleolar (AC-8, 9, 10) or Centromere (AC-3)	Scleroderma Panel	Scl-70, Centromere, RNA Poly III	RNA Polymerase III is frequently absent from standard ENA kits.
Lupus Erythematosus	Homogeneous (AC-1) or Speckled (AC-4, 5)	ENA Panel + anti-dsDNA	Sm, RNP, SSA, SSB	Anti-Sm is highly specific but may present at low titers.
Autoimmune Hepatitis	Homogeneous (AC-1)	Liver Autoimmunity Panel	Anti-smooth muscle (actin), LKM1, SLA	Targets are absent from standard ENA panels (ANA+/ENA- discrepancy).

Decision-Making Strategy: From Concordance to Clinical Exceptions

1. Concordant Scenarios: Optimizing Diagnostic Efficiency

In most cases, laboratory findings corroborate the clinical presentation. The primary challenge lies in using IIF parameters to effectively guide second-line testing:

- **The Value of Antibody Titers:** Titers reflect autoantibody concentration. Low titers (e.g. 1:80) are common in healthy individuals or as incidental findings in asymptomatic patients, warranting simple monitoring. Conversely, high titers significantly increase the probability of detecting anti-ENA and justify comprehensive follow-up testing (3).
- **The Utility of Staining Patterns (ICAP Nomenclature):** Qualitative assessment refines diagnostic hypotheses. Homogeneous and speckled patterns typically point toward classical nuclear antigens (SSA, Sm, RNP). In contrast, nucleolar or cytoplasmic patterns are strong indicators of specific entities such as systemic sclerosis or inflammatory myopathies (2).

2. Discordant Scenarios: Prioritizing Clinical Judgment Over Biology

Clinicians must be prepared to deviate from standard algorithms to avoid the pitfalls of under- or over-diagnosis:

- **Indications for anti-ENA despite a Negative ANA:** While IIF has a high negative predictive value, it should not halt investigations when clinical suspicion is high. Anti-ENA testing is mandatory in cases of suspected subacute lupus, Sjögren's syndrome, systemic Raynaud's phenomenon, inflammatory myopathy, or unexplained cytopenias (1).
- **Clinical Abstention despite a Positive ANA:** A positive ANA result does not systematically justify the use of an ENA panel, especially if the titer is low, the finding is incidental, or the clinical context suggests transient inflammation. This measured

approach prevents the reporting of non-specific results that generate patient anxiety and lead to unnecessary medical workups (1).

Summary: An Optimized Decision-Making Algorithm (figure1)

This diagnostic approach follows three key stages:

1. Initial Clinical Assessment

The clinical utility of ANA testing depends entirely on the presence of suggestive symptoms (e.g., skin involvement, inflammatory arthralgia, sicca syndrome, or renal symptoms). In the absence of clinical suspicion, routine screening is discouraged to minimize the incidence of incidental findings.

2. Nuanced Interpretation

Diagnostic orientation should not rely on raw results alone, but on the intersection of titer, staining pattern, and pre-test probability (11):

- **Negative or low-titer ANA with low clinical suspicion:** The negative predictive value of IIF is excellent. To avoid overdiagnosis, we recommend halting investigations and opting for simple clinical monitoring.
- **Negative ANA with high clinical suspicion:** IIF should not act as a diagnostic bottleneck. In a typical clinical presentation (e.g., Sjögren's syndrome), ordering a targeted anti-ENA panel—specifically anti-SSA—is essential.
- **Intermediate to high-titer ANA or suggestive patterns:** The probability of an underlying systemic CTD is significant. An anti-ENA panel tailored to the observed ICAP pattern is required.

3. Anti-ENA Integration and Final Validation

The identification of an antigenic specificity must strictly correlate with the clinical presentation. Interpretation follows two rigorous principles:

- **Primacy of Symptoms:** An isolated antibody, without symptomatic correlation, is insufficient to diagnose a systemic connective tissue disease.
- **Evolutionary Vigilance:** Conversely, the absence of anti-ENA does not rule out early-stage disease or incomplete phenotypes. Periodic clinical and biological reassessment is necessary based on the patient's progression.

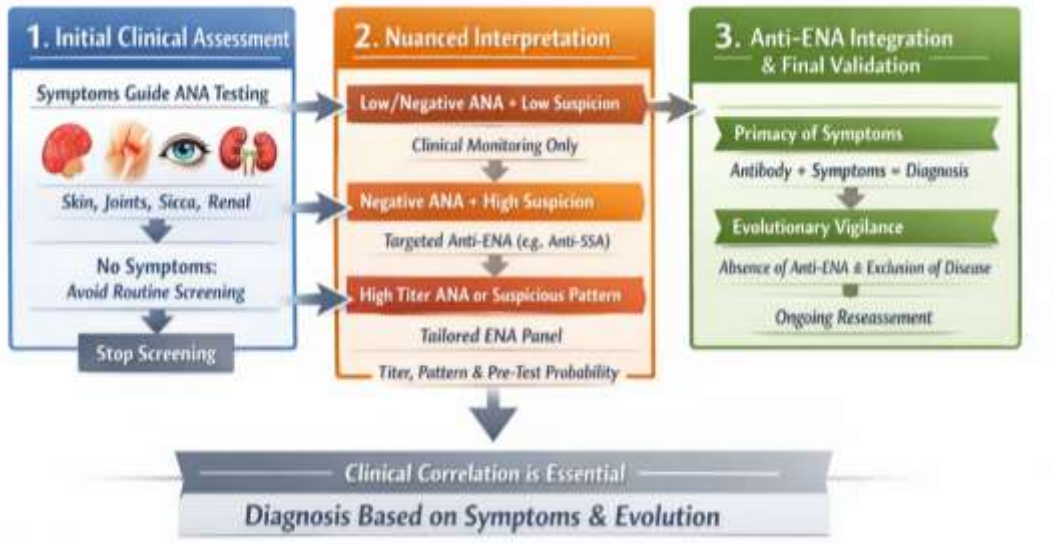

Figure 1: An Optimized Decision-Making Algorithm

Key Takeaways for Clinical Practice

- **Screening Utility:** ANA remains an excellent primary screening tool for systemic autoimmune diseases.
- **Clinical Relevance:** The diagnostic value of an ANA result is strictly dependent on both its titer and its staining pattern.
- **Technical Awareness:** Discrepancies between ANA and anti-ENA results are not uncommon and are often explained by the inherent technical limitations of indirect immunofluorescence.
- **The "Negative ANA" Exception:** A negative ANA result does not definitively rule out systemic pathology when clinical suspicion is high (e.g. isolated anti-SSA in Sjögren's syndrome).
- **Risk of Overdiagnosis:** An isolated positive result at a low titer is insufficient, on its own, to establish a diagnosis of systemic connective tissue disease.
- **Algorithmic Rigor:** The sequential diagnostic algorithm remains highly effective, provided it is applied within a rigorous clinical framework.

CONCLUSION

The "ANA followed by anti-ENA" algorithm remains a rational and efficient strategy for investigating systemic autoimmune diseases. However, its success depends on its integration with sound clinical reasoning and a nuanced

interpretation of serological parameters. Understanding the mechanisms behind ANA/ENA discrepancies is essential to prevent interpretative errors while optimizing diagnostic accuracy. Beyond the strict adherence to an algorithm, the ultimate goal remains a reasoned prescription approach that serves the interests of precision medicine.

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