Discordant detection of anti-centromere antibodies by indirect immunofluorescence and ELISA: A case report and diagnostic considerations

Running Title: ACA discordance: IIF vs ELISA

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Abstract

Background: Anti-centromere antibodies (ACA) are commonly detected by indirect immunofluorescence (IIF) on HEp-2 cells, typically producing the classic centromere pattern (AC-3). However, discrepancies between IIF and solid-phase immunoassays such as ELISA may complicate interpretation.

Case Presentation: We report the case of a 75-year-old woman presenting with progressive inflammatory polyarthritis, morning stiffness, dry mouth, photosensitivity, and mild exertional dyspnea. Antinuclear antibody (ANA) testing by IIF on HEp-2 cells demonstrated a multiple nuclear dots pattern (AC-6) with features resembling a CENP-F–like pattern (AC-14), rather than the typical centromere pattern. In contrast, solid-phase assays, including ELISA and immuno-dot blot, demonstrated anti-centromere antibody reactivity. Additional serologic findings included strong positivity for SSA/Ro60, Ro52/TRIM21, SSB/La, and DFS70 antibodies.

Conclusion: This case demonstrates that anti-centromere reactivity detected by solid-phase assays may occur despite the absence of a classic AC-3 pattern on IIF. Interpretation of discordant serologic findings requires careful integration of clinical features with complementary laboratory methods.

Keywords: Anti-centromere antibodies; HEp-2; Indirect immunofluorescence; ELISA; ANA patterns; CENP-F

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Introduction

Anti-centromere antibodies (ACA) target specific centromere proteins, most notably CENP-A, CENP-B, and CENP-C, and are considered highly characteristic markers of systemic sclerosis, especially the limited cutaneous form (1,2). Indirect immunofluorescence (IIF) on HEp-2 cells remains the principal screening method for antinuclear antibodies, in which ACA typically produces the well-recognized centromere pattern (AC-3) (1,4,7,10). As solid-phase immunoassays such as ELISA have become more widely used in routine diagnostic practice, situations in which IIF findings and antigen-specific test results do not correspond have been reported with increasing frequency (3). Discordant ANA findings between indirect immunofluorescence and solid-phase immunoassays are increasingly recognized in routine clinical practice and may present significant interpretative challenges (9). In this report, we present a case exemplifying this issue and explore the methodological and diagnostic considerations that arise.

Case presentation

A 75-year-old woman was referred for evaluation because of progressive fatigue and inflammatory joint pain. Over the preceding six months, she had experienced worsening morning stiffness lasting more than one hour, pain and swelling involving both wrists and multiple metacarpophalangeal joints, and progressive reduction in hand function. She also reported dry mouth, intermittent photosensitivity, and mild exertional dyspnea. Fever, weight loss, skin rash, and Raynaud phenomenon were absent.

Her past medical history included hypertension and hyperlipidemia, treated with amlodipine and atorvastatin. There was no history of chronic infection, hepatitis, malignancy, or previously diagnosed autoimmune disease. Family history was significant for rheumatoid arthritis in her sister.

On examination, the patient was afebrile with stable vital signs. Synovitis was present in both wrists and several metacarpophalangeal joints. No sclerodactyly, digital ulcers, lymphadenopathy, or hepatosplenomegaly were identified.

Because the clinical findings suggested chronic inflammatory polyarthritis with possible systemic autoimmune involvement, a comprehensive immunologic assessment was requested.

Laboratory findings

Table 1 summarizes the patient's serological and immunological laboratory findings. The results demonstrated positive anti-centromere antibody reactivity on solid-phase testing together with strong positivity for SSA/Ro60, Ro52/TRIM21, SSB/La, and DFS70 antibodies. In contrast, ANA indirect immunofluorescence showed an atypical multiple-nuclear-dot pattern rather than the classic centromere pattern, highlighting the discordance between IIF and antigen-specific assays.

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Table 1. Laboratory findings of patient

Test	Result	Interpretation
Multiple Nuclear Dots Pattern		
Anti-centromere antibody (Dot blot)	Positive	Abnormal
SSA/Ro60	Strong positive	Abnormal
Ro52/TRIM21	Strong positive	Abnormal
SSB/La	Strong positive	Abnormal
DFS70	Positive	Abnormal
Anti-RNP	Negative	Normal
Anti-MCV	Negative	Normal
Serologic Study		
ANA by IIF on HEp-2 cells	Positive	Abnormal
Serum IgG4	Within normal range	Normal
Complement C3/C4	Within normal range	Normal
ANCA (PR3/MPO)	Negative	Normal
Cryoglobulin	Negative	Normal

ANA testing was initially performed by indirect immunofluorescence (IIF) using HEp-2 cell substrate slides (Euroimmun AG, Lübeck, Germany). Initial IIF evaluation demonstrated a speckled nuclear pattern. However, subsequent immuno-dot blot testing revealed anti-centromere reactivity, creating a discordance between the observed IIF pattern and antigen-specific assay results. Consequently, the specimen was referred to our reference laboratory for further evaluation. ANA interpretation in the reference laboratory was performed using the automated AKLIDES® system (Medipan GmbH, Dahlewitz, Germany). ENA and autoantibody testing were conducted using ELISA and immuno-dot blot assays according to the manufacturers' instructions (4, 5).

In our laboratory, repeat ANA testing demonstrated numerous discrete nuclear dots in interphase nuclei without the characteristic chromosomal staining expected in a classic centromere pattern (ICAP AC-3). Some cells also displayed patchy perinuclear and spindle-associated staining, suggesting overlapping atypical centromere/CENP-F–like features consistent with ICAP AC-14 (4–6). Repeat IIF testing using a different HEp-2 substrate lot again demonstrated a multiple nuclear dots pattern (AC-6) without a classic centromere (AC-3) pattern, further supporting the presence of atypical centromere/CENP-F–like reactivity.

Consideration of a CENP-F–like (AC-14) pattern

A CENP-F–like ANA pattern was considered because staining on HEp-2 cells showed features that varied with the cell cycle, including subtle punctate staining confined mainly to prometaphase and metaphase cells, together with occasional weak nuclear-envelope and mid-body reactivity (4–6). The ICAP initiative has emphasized the importance of standardized interpretation and reporting of HEp-2 IIF patterns in autoimmune serology (11). However, in this patient, the presence of high-titer SSA/Ro60, Ro52, SSB/La, and DFS70 antibodies, combined with detectable anti-centromere reactivity on two solid-phase platforms, suggests that the AC-14 component may represent a concurrent pattern rather than the principal source of disagreement between IIF and ELISA. Demonstrating true CENP-F specificity would require confirmatory testing by targeted immunoblot or line-blot analysis, together with reproducible mitotic-cell–predominant staining on repeat IIF.

Reports describing AC-14 indicate that sera with this pattern are frequently identified in patients with various malignancies, although its overall prevalence remains low, even in oncologic cohorts (1–3). AC-14 has also been reported in inflammatory and immune-mediated

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disorders, including autoimmune liver disease, Crohn's disease, Sjögren's syndrome, and graft-versus-host disease. In the present case, no clinical or laboratory evidence of malignancy was identified. Because current evidence is derived largely from case reports and small series, the clinical significance of AC-14 remains incompletely defined, and specific commercial immunoassays for CENP-F antibodies are not currently available (3–7).
Several mechanisms may account for the discordance observed between IIF and solid-phase testing in this case.

1. **Differences in analytical sensitivity.** ELISA and dot-blot platforms target isolated centromere proteins, most commonly CENP-B, and may detect low-titer antibodies that fail to produce the characteristic AC-3 pattern on HEp-2 cells (1, 3).
2. **Epitope conformation.** IIF recognizes antibodies binding native nuclear antigens in situ, whereas solid-phase systems rely on recombinant or purified antigens; antibodies to linear or cryptic epitopes may react strongly on ELISA but show minimal binding in intact cells (1, 3).
3. **Interfering or dominant autoantibodies.** Multiple concurrent antibodies—particularly SSA/Ro, Ro52, SSB/La, and DFS70—can generate prominent fluorescence that masks or modifies underlying AC-3 staining, complicating interpretation (2, 5).

Previous studies have similarly emphasized that anti-centromere antibody assays may demonstrate variable concordance across different analytical platforms depending on antigen composition and epitope recognition (8). In this case, both ELISA and immuno-dot blot confirmed anti-centromere reactivity, yet the absence of a classic AC-3 pattern suggests that antigen exposure, antibody avidity, or epitope specificity may explain the divergence.
From a clinical perspective, the overall antibody profile—ACA positivity coexisting with strong SSA/Ro52/SS-B and DFS70—supports cautious longitudinal monitoring for evolving connective-tissue or overlap syndromes, rather than attributing the findings to a single diagnostic entity (Figure 2).

Conclusion

This case demonstrates that anti-centromere reactivity detected by ELISA or dot-blot assays may occur even when the classic AC-3 centromere pattern is absent on ANA IIF. Rather than representing technical error, this type of discrepancy likely reflects inherent differences in antigen presentation and analytical behavior across testing platforms. Interpreting such results therefore requires careful integration of clinical information with findings from multiple serologic methods, and repeat testing may be appropriate when uncertainty persists. Clinicians and laboratory specialists should avoid assigning diagnostic significance to isolated solid-phase positivity in the absence of corroborating IIF patterns or compatible clinical manifestations.

Ethics Statements

The patient provided informed consent for publication of this case report. The study was conducted in accordance with institutional and ethical guidelines.

Consent for Publication

Written informed consent for publication was obtained from the patient.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

Data supporting the findings of this study are available from the corresponding author upon reasonable request.

Author Contributions

M.S.S. designed the study, interpreted laboratory findings, and prepared the manuscript. O.S.S. contributed to clinical interpretation. G.A. and M.F. participated in laboratory investigations and technical review. A.G. contributed to scientific revision and final manuscript review. All authors approved the final version.

References

1. Xiong J, et al. Anti-centromere antibodies in a large cohort of systemic sclerosis patients: comparison between immunofluorescence, CENP-A and CENP-B ELISA. *Arthritis Rheum*. 2011;63:1479-1488.
2. Smith V, et al. Clinical and serological heterogeneity in patients with anticentromere antibodies. *Arthritis Res Ther*. 2005;7:R1060-R1070.
3. Zhao Y, et al. Correlation among different anti-centromere assays and HEp-2 IIF patterns. *Clin Chim Acta*. 2018;481:39-46.
4. Fritzler MJ, Chan EKL. Clinical relevance of HEp-2 IIF patterns including rare patterns. *Autoimmun Rev*. 2015;14:967-974.
5. International Consensus on ANA Patterns (ICAP). AC-14 CENP-F–like pattern. Available from: https://www.anapatterns.org/view_pattern.php?pattern=14 [Accessed 2026 May 29].
6. Lee JH, et al. Centromere protein-F–like pattern in a patient with rheumatoid arthritis. *Clin Exp Rheumatol*. 2018;36 Suppl 112:133-5.
7. Sheikh Sajjadieh M, Ajami A, Haghshenas L. Estimation of sensitivity and specificity of antinuclear antibody by automated indirect immunofluorescence and enzyme-linked immunoassay. *mljgoums*. 2024;18(4):22-3.
8. Mahler M, Fritzler MJ. The clinical significance of the anti-centromere antibody assay in systemic autoimmune rheumatic diseases. Autoimmun Rev. 2021;20(8):102912.
9. Infantino M, Meacci F, Grossi V, et al. The burden of discordant anti-nuclear antibody testing in clinical practice. Diagnostics (Basel). 2022;12(5):1148.
10. Damoiseaux J, Andrade LEC, Fritzler MJ, et al. Clinical relevance of HEp-2 indirect immunofluorescent patterns: the International Consensus on ANA Patterns (ICAP) perspective. Ann Rheum Dis. 2019;78(7):879-89.
11. Chan EKL, Damoiseaux J, de Melo Cruvinel W, et al. Report of the first International Consensus on standardized nomenclature of antinuclear antibody HEp-2 cell patterns 2014–2015. Front Immunol. 2015;6:412.

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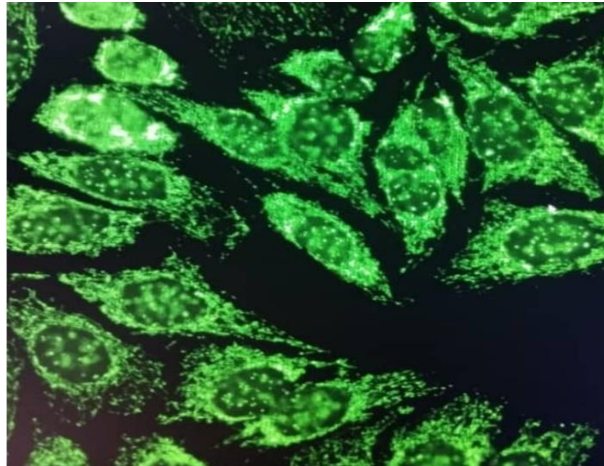

Figure 1. Indirect immunofluorescence on HEp-2 cells demonstrating a multiple nuclear dots pattern (ICAP AC-6) with overlapping atypical centromere/CENP-F-like features (ICAP AC-14). Mitotic cells lacked the characteristic chromosomal staining of a classic centromere pattern (AC-3).

Antigen	Intensity	Class
RNP/Sm (RNP/Sm)	2	o
Sm (Sm)	2	o
SS-A native (60 kDa) (SSA)	77	+++
Ro-52 recombinant (52)	106	+++
SS-B (SSB)	62	+++
Scl-70 (Scl)	2	o
PM-Scl100 (PM100)	17	+
Jo-1 (Jo)	2	o
Centromere B (CB)	86	+++
PCNA (PCNA)	3	o
dsDNA (DNA)	0	o
Nucleosomes (NUC)	0	o
Histones (HI)	0	o
Ribosomal Protein (RIB)	3	o
AMA-M2 (M2)	2	o
DFS70 (DFS70)	11	+
Control (Ko)	95	+++
Label (ET)	-1	

Figure 2. ENA/ANA profile showing antigen-specific assay results discordant with IIF findings. The line immunoassay demonstrates strong reactivity to SSA/Ro60 and moderate positivity to SSB and PM-Scl100, with negative anti-CENP-B despite an IIF pattern initially suggestive of centromere staining. DFS70 is weakly positive. These findings illustrate a clinically relevant discrepancy between IIF pattern recognition and antigen-specific assays.