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Performance of anti-topoisomerase 1 antibody testing by multiple-bead, enzyme-linked immunosorbent assay, and immunodiffusion in a university setting

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Abstract

Background/Objective: The gold standard for anti- topoisomerase I antibody (anti-topo I antibody) testing in systemic sclerosis (SSc) uses immunodiffusion (ID) techniques, but enzyme-linked immunosorbent assay (ELISA) and multi-bead technology are often used in current settings to save time and cost. Our aim was to assess the performance of the multi-bead, ELISA, and ID testing methods.

Methods: We conducted a retrospective study of patients at the University of Michigan whose extractable nuclear antigen-10 (ENA-10) autoantibody panel tested positive for the anti-topo I antibody by multi-bead technology during a one-year period. All samples positive by multi-bead were sent to the RDL Laboratories and reflexed for ELISA, and all anti-topo I antibodies positive by ELISA were further tested by ID. Clinical data was reviewed by a rheumatologist and assessed for presence of SSc. Data was analyzed via frequency tables.

Results: Approximately 9,500 ENA-10 panels were ordered by physicians at the University of Michigan. Of these, 129 patients were positive for the anti-topo I antibody by multi-bead assay, 51 were positive by multi-bead assay and ELISA, and 21 were positive by multi-bead assay, ELISA, and ID. We found that 26.4% of patients positive by multi-bead, 47.1% positive by multi-bead assay and ELISA, and 95.2% positive by multi-bead assay, ELISA and ID had SSc.

Conclusion: Multi-bead assays have a high rate of false positive results for the anti-topo I antibody in patients without clinical evidence of SSc. A stepwise approach of confirmation of positive multi-bead results using both ELISA and ID improves the predictive value of antibody testing for the diagnosis of SSc.

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Keywords

systemic sclerosis; immunodiffusion; enzyme-linked immunosorbent assay; anti-Scl-70; anti-topoisomerase I

INTRODUCTION

Systemic Sclerosis (SSc) is a rare autoimmune disease which affects the connective tissue of the skin and internal organs. SSc can be heterogeneous, ranging from minimal to severe skin involvement and may affect the internal organs. SSc has a higher morbidity and mortality than any other rheumatic disease and affects an estimated 240 people per million in the United States.[1, 2]

Classification of SSc is based on the 2013 European League Against Rheumatism (EULAR)/American College of Rheumatology (ACR) classification criteria.[3] These criteria include signs, symptoms and assessment of three SSc-related autoantibodies: anti-centromere, anti-topoisomerase I (anti-topo I, also known as anti-Scl-70) and anti-RNA polymerase III. In the United States, anti-topo I antibody has been found in about 20% of patients with SSc.[4, 5] The presence of anti-topo I antibody is associated with an increased risk of developing diffuse cutaneous SSc (dcSSc), scleroderma renal crisis, and scleroderma-related progressive interstitial lung disease (ILD).[4, 6] In the United States, about 30–40% of dcSSc patients are positive for the anti-topo I antibody compared with approximately 10–20% of limited cutaneous SSc (lcSSc) patients.[7–9] Sensitivity and specificity of the anti-topo I antibody test for a diagnosis of SSc has been reported at 20–40% and 90–100%, respectively,[10–12] while sensitivity and specificity of anti-topo I antibody for the dcSSc subgroup has been reported at 40–60% and 95%, respectively.[11, 13]

Current laboratory testing for the anti-topo I antibody varies by institution and includes multiplex magnetic bead technology (multi-bead), enzyme-linked immunosorbent assay (ELISA), and immunodiffusion (ID). The gold standard for anti-topo I antibody testing uses immunodiffusion (ID) techniques, however, multi-bead testing is the most prevalent in clinical settings as they are automated and therefore are less time consuming. The multi-bead testing method allows multiple analytes to be measured in a single run of the assay, which results in the advantages of increased efficiency and reduced expense.[14] However, there has been concern that using this methodology causes increased false positivity of the anti-topo I antibody.

Our aim was to assess the performance of the multi-bead, ELISA, and ID testing methods for anti-topo I antibody within a single academic center.

METHODS

We conducted a retrospective study of 129 patients at the University of Michigan whose extractable nuclear antigen-10 (ENA-10) autoantibody panel tested positive for anti-topo I antibody by multi-bead technology during a one-year period from August 2016 to August 2017. Ethics board approval from the University of Michigan Internal Review Board

(IRBMED) (HUM00142710) with a waiver of informed consent for secondary use of existing identifiable data was obtained. Anti-topo I antibody testing at the University of Michigan Clinical Immunology Laboratory is performed using the BioPlex 2200 system. This system employs heterogeneous sets of 8μm-diameter magnetic beads infused with varying ratios of two fluorescent classification dyes, creating a series of unique bead sets. Beads within each set are coated with a single purified ligand specific to the particular assay, allowing the capture and detection of corresponding specific analytes from a clinical sample. Target analytes captured on bead surfaces are in turn probed with a corresponding fluorescent conjugate. With excitation and emission spectra distinct from those of the classification dyes used to identify analyte and control beads, the conjugate serves as the "reporter" fluorescence signal.

In this study, all samples positive for the anti-topo I antibody by multi-bead were sent to the RDL Reference Laboratory to be reflexed for ELISA, and all anti-topo I antibodies positive by ELISA were further tested by ID. Anti-topo I antibody ELISA testing was performed on the QUANTA Lite® Scl-70 ELISA assay (Inova Diagnostics, San Diego, CA). Anti-topo I antibody testing by ID was performed by a proprietary procedure using an anti-topo I antigen from Inova Diagnostics.

In an additional 24 patients who were positive for anti-topo I on the multi-bead platform, we reviewed the values in International Units (IU) and assessed if the values were 1–8 IU or > 8 IU (above the measurement value) and its relationship with the diagnosis of SSc and other CTDs.

Clinical data for all patients was reviewed by the first author and a rheumatologist (D.K.). We assessed if the patients were seen in a rheumatology clinic, if they fulfilled the 2013 ACR/EULAR classification criteria for SSc, and if a diagnosis of SSc was established. If not, we also reviewed if a diagnosis of another connective tissue disease (CTD) was made. For those who were not referred to rheumatology clinic, the charts were reviewed for signs, symptoms, and other autoantibodies suggestive of a CTD. We also documented evidence of internal organ involvement (interstitial lung disease (ILD), gastroesophageal disease (GERD), scleroderma renal crisis, or pulmonary hypertension). SSc disease duration was determined using the onset date of the patient's initial non-Raynaud's phenomenon sign or symptom. Data was analyzed for predictive values and likelihood ratios using frequency tables.

RESULTS

During the period of one year, approximately 9,500 ENA panels were ordered by physicians at University of Michigan. ENA panel were generally ordered by non-rheumatologists or rheumatologists who considered an underlying CTD. Among these, 129 (1.4%) patients had positive anti-topo I antibody by multi-bead assay and of these 129 patients, 51 (39.5%) were positive by ELISA. Of those patients positive by ELISA, 21 (41.2 %) were positive by ID (Table 1).

On chart review of the patients who were positive by multi-bead assay, 34 (26.4%) had SSc and 9 (26.5%) of these 34 had dcSSc. Twenty-three (17.8%) had other CTDs, with 72 (55.8%) presenting with no evidence of CTD (Table 1). Of the 72 presenting with no evidence of CTD, 41 were evaluated by a rheumatologist.

Of the 51 patients who were positive by multi-bead and ELISA, 24 (47.1%) had a diagnosis of SSc and 8 of these 24 (33.3%) had dcSSc. Seven (13.7%) were diagnosed with other CTDs (Table 2). For the 21 patients who were positive by multi-bead, ELISA and ID, 20 (95.2%) were diagnosed with SSc and 8 of these 20 (40.0%) had dcSSc. Of the 20 patients with SSc, 15 (75.0%) had evidence of internal organ involvement with the majority of the organ involvement including GERD and/or ILD (Table 3). ELISA units for those positive by ID ranged from 32–231 units. Fourteen (93.3%) of those with values equal to or greater than 110 units were diagnosed with SSc. ELISA units for those positive by ID with a diagnosis of dcSSc ranged from 70–129 units, and 6 of these 8 patients had values above 110 units.

The positive predictive value (PPV) for the diagnosis of SSc was 47.1% by multi-bead and ELISA and 95.2% by multi-bead, ELISA, and ID and the PPV for diagnosis of dcSSc was 15.7% by multi-bead and ELISA and 38.1% by multi-bead, ELISA, and ID. The positive likelihood ratio (LR) for the diagnosis of SSc was 2.5 (negative LR=0.4) and 2.5 (negative LR=0.2) for the diagnosis of dcSSc by multi-bead and ELISA. The positive LR for the diagnosis of SSc was 11.7 (negative LR 0.2) and 3.3 (negative LR=0.0) for dcSSc by multi-bead, ELISA, and ID.

In an additional 24 patients who were positive for the anti-topo I antibody by multi-bead between May 2018 and July 2018 and had availability of the fluorescence signal (reported as International Units (IU)) for the multi-bead assay, we found that 4 (16.7%) had IU values of >8.0. All 4 of these were positive by both ELISA and ID, 2 (50.0%) had dcSSc, 1 (25.0%) had lcSSc, and 1 (25.0%) had early undifferentiated connective tissue disease (UCTD) with Raynaud's phenomenon. No subjects whose IU values were below 8.0 had dcSSc; 2 (10.0%) had lcSSc, 2 (10.0%) had UCTD and 3 (15.0%) had other CTDs.

DISCUSSION

Anti-topo I antibody testing is widely used to assist in the diagnosis of SSc and to identify individuals at risk for progressive disease with internal organ involvement. Traditionally, anti-topo I antibody has been utilized due to its perceived high specificity in diagnosing SSc.[10, 11] The gold standard to evaluate anti-topo I antibodies is the ID method, but ID is difficult to automate and requires 2–3 days to process.[12] Multi-bead assay was introduced to save time, material and labor costs and to increase the efficiency of autoantibody testing.[14] This methodology has been largely utilized in the United States healthcare system, but our results suggest a high rate of false positives for anti-topo I by multi-bead assay, leading to additional testing, inappropriate referral to rheumatologists, and consternation among patients. We believe that our stepwise approach is cost-effective and may help to streamline the diagnosis and care of patients with true positive anti-topo I antibodies, having been verified by either ELISA or ID.

ID has been traditionally used due to its high specificity for the diagnosis of SSc.[10] When determined by ID, anti-topo I antibodies are rarely seen in healthy controls, non-affected relatives of patients with SSc, or in patients with other CTDs or primary Raynaud's phenomenon.[12] Literature on non-ID techniques show a lower clinical specificity, especially in rheumatic disease controls.[10] Differences in epitope recognition, manner of antigen/epitope display on bead surface, and/or antibody avidity and affinity in solid-phase and liquid-phase assays may explain this discrepancy.[15] Others suggest that the false positive results may be due to contamination of antigens or binding of anti-DNA/DNA complexes to topoisomerase-I.[5]

Mahler et al[5] reviewed the literature for association of anti-topo I antibody in those who were diagnosed with SLE. The presence of anti-topo I antibody by ELISA, multi-bead, and other methodologies ranged from 0.0% to 7.7%.[5, 13, 16–23] Using BioplexTM 2200 assay, it ranged from 1.2% to 5.2%.[5, 16, 17] The prevalence was higher in SLE compared to healthy controls in their review. In our cohort, 3.9% had SLE who were positive on multi-bead assay (Table 1).

Screening for the anti-topo I antibody by BioPlex2200 (multi-bead) and ELISA is set to minimize false negatives, though it has been found that patients with SSc tend to have higher anti-topo I antibody titers than healthy donors or patients with SLE.[5, 15] We explored if the fluorescence signal on multi-bead and ELISA titers were associated with likelihood of SSc in additional 24 patients and found that higher values were likely associated with SSc. However, only 3 of 4 patients with high titers had a diagnosis of SSc and remaining had a diagnosis of UCTD.

Our study has several strengths. First, we followed a stepwise approach that was instituted prospectively with RDL. Second, we reviewed all charts individually and were able to determine whether the patients had CTD due to SSc or other CTDs. Lastly, our step-wise approach is cost-effective as it only sends specimens that are positive by multi-bead assay to RDL lab for further testing. In the current study, 1.4% of the samples were sent to the RDL lab for confirmation.

Our study also has limitations. The study only included patients who were positive by multi-bead testing, and tests negative by ELISA were not evaluated further by ID. Therefore, we were unable to calculate true sensitivity and specificity for each test, although these have been evaluated elsewhere.[10, 13, 24, 25] Second, we did not assess if there is a cut-point on the fluorescence signal for the multi-bead assay on every patient but explored an additional 24 subjects who had recent laboratory work. In addition, all samples were only sent to a single laboratory (RDL); laboratories differ in their testing for autoantibodies, and this needs to be confirmed in other commercial and/or research laboratories.

In summary, we demonstrate that multi-bead assays have a high rate of false positive tests for the anti-topo I antibody in patients without any clinical evidence of SSc. A stepwise approach of confirmation, using both ELISA and ID, greatly improves the predictive value of antibody testing for the diagnosis of SSc, and helps exclude other scleroderma variants and/or other CTDs.

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Table 1:

Relative frequency of anti-topo I antibody in patients diagnosed with systemic sclerosis, diffuse cutaneous systemic sclerosis, systemic lupus erythematosus, and other connective tissue diseases

	Total n (%)	Systemic Sclerosis n (%)	Diffuse Cutaneous Systemic Sclerosis n (%)	Systemic Lupus Erythematosus n (%)	Other CTDs n (%)
Anti-topo I positive by multi-bead	129 (100)	34 (26.4)	9 (7.0)	5 (3.9)	18 (14.0)
Anti-topo I positive by multi-bead and ELISA	51 (39.5)	24 (47.1)	8 (15.7)	2 (3.9)	5 (9.8)
Anti-topo I positive by multi-bead, ELISA and ID	21 (41.2)	20 (95.2)	8 (38.1)	0 (0.0)	0 (0.0)

Table 2:

Relationship of connective tissue diseases by multi-bead assay, ELISA and immunodiffusion

	Anti-topo I positive by multi-bead	Anti-topo I positive by multi-bead and ELISA	Anti-topo I positive by multi-bead, ELISA & ID
Sjogren's Syndrome	4	0	0
Systemic Lupus Erythematosus	5	2	0
Rheumatoid Arthritis	6	3	0
Undifferentiated Connective Tissue Disease	2	0	0
Inflammatory Polyarthritis	1	0	0
Dermatomyositis & Clinically Amyopathic Dermatomyositis	2	1	0
Eosinophilic Faciitis	1	0	0
Polymyalgia Rheumatica	1	0	0
Seronegative Inflammatory Arthritis	1	1	0

Table 3:

Disease duration and organ involvement in SSc patients positive by multi-bead assay, ELISA, and ID for anti-topo I (n=21)*

	Systemic Sclerosis	Limited Cutaneous and Sine Systemic Sclerosis**	Diffuse Cutaneous Systemic Sclerosis
N (%)	20 (95.2)	12 (57.1)	8 (38.1)
Disease Duration, years (SD)	4.9 (4.4)	3.7 (3.1)	4.9 (6.9)
Interstitial Lung Disease	10 (47.6)	5 (41.7)	5 (62.5)
Gastroesophageal Reflux Disease	13 (61.9)	7 (58.3)	6 (75.0)
Scleroderma Renal Crisis	0 (0.0)	0 (0.0)	0 (0.0)
Pulmonary Hypertension	4 (19.0)	1 (8.3)	3 (37.5)

* One patient with positive anti-topo I antibody by ID did not have a diagnosis of SSc and had primary Raynaud's disease.

** Four of this group had Sine Scleroderma.