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

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INTRODUCTION

- Systemic Sclerosis (SSc) is a rare autoimmune disease which affects the connective tissue of the skin and internal organs
- In the United States, anti-topo I antibody has been found in about 20% of patients with SSc.[1]
- 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).[1,2]
- The gold standard for anti- topo I antibody testing is immunodiffusion (ID).
- Enzyme-linked immunosorbent assay (ELISA) and multi-bead technology are often used in current settings to save time and cost.
- There has been concern that using this methodology causes increased false positivity of the anti-topo I antibody.
- Others have postulated that the 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.[3]
- Contamination of antigens or binding of anti-DNA/DNA complexes to topo-I may also account for this.[4]

OBJECTIVE

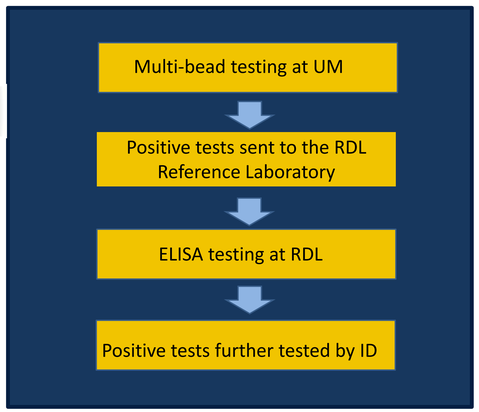
Assessment of anti-topo I antibody testing at the University of Michigan

- 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

Step-wise antibody testing method

- 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.
- Anti-topo I antibody testing at UM is performed via the multi-bead method using the BioPlex 2200 system.
- All samples positive for the anti-topo I antibody by multi-bead testing were sent to the RDL Reference Laboratory for further testing by ELISA, and if positive, by ID.
- Anti-topo I ELISA testing was performed on the QUANTA Lite® Scl-70 ELISA assay (Inova Diagnostics, San Diego, CA).
- Anti-topo I 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, we reviewed the multi-bead values in International Units (IU) and its relationship with the diagnosis.



METHODS

Clinical Data

- 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 or other connective tissue disease (CTD) was established.
- 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).

RESULTS

- During the period of one year, approximately 9,500 ENA panels were ordered by physicians at UM.
- 129 (1.4%) patients had positive anti-topo I antibody by multi-bead assay.
- Of those patients positive by multi-bead, 51 (39.5%) were positive by ELISA.
- Of those patients positive by multi-bead and ELISA, 21 of 51 (41.2 %) were positive by ID (Table 1).

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 (%)	SSc n (%)	dcSSc n (%)	SLE 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 + 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)

*One patient with positive anti-topo I antibody by ID had primary Raynaud's phenomenon without an associated CTD.

RESULTS

SSc and CTD Diagnoses

- Of the 129 patients positive by multi-bead, 34 (26.4%) had SSc and 9 (26.5%) of these 34 had dcSSc
- 23 (17.8%) had other CTDs
- 72 (55.8%) presented with no evidence of CTD. (Table 1)
- 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.
- 7 (13.7%) were diagnosed with other CTDs. (Table 2)

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 ELISA
Sjogren's Syndrome, n (%)	4 (17.4)	0 (0.0)
Systemic Lupus Erythematosus, n (%)	5 (21.7)	2 (28.6)
Rheumatoid Arthritis, n (%)	6 (26.1)	3 (42.9)
Undifferentiated Connective Tissue Disease, n (%)	2 (0.9)	0 (0.0)
Inflammatory Polyarthritis, n (%)	1 (4.3)	0 (0.0)
Dermatomyositis & Clinically Amyopathic Dermatomyositis, n (%)	2 (8.7)	1 (14.3)
Eosinophilic Faciitis, n (%)	1 (4.3)	0 (0.0)
Polymyalgia Rheumatica, n (%)	1 (4.3)	0 (0.0)
Seronegative Inflammatory Arthritis, n (%)	1 (4.3)	1 (14.3)

*No patients who were anti-topo I positive by multi-bead, ELISA and ID had CTDs.

- For the 21 patients who were positive by multi-bead, ELISA and ID, 20 (95.2%) were diagnosed with SSc and 8 (40.0%) of these 20 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.
- In an additional 24 patients who were positive for the anti-topo I antibody by multi-bead 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). (Table 3)

RESULTS

Table 3: Relative frequency of anti-Scl-70 antibody in 24 additional patients with multi-bead IU values

	Total n (%)	DcSSc n (%)	LcSSc* n (%)	UCTD n (%)	Other CTDs n (%)
Anti-topo I positive by multi-bead	24 (100)	2 (8.3)	2 (8.3)	4 (16.7)	3 (12.5)
Anti-topo I Positive by multi-bead + ELISA	14 (58.3)	2 (14.3)	1 (7.1)	4 (28.6)	3 (21.4)
Anti-topo I Positive by multi-bead, ELISA and ID**	4 (16.7)	2 (50.0)	0 (0.0)	2 (50.0)	0 (0.0)
IU values ≥ 8.0	4 (16.7)	2 (50.0)	1 (25.0)	1 (25.0)	0 (0.0)
IU values < 8.0	20 (83.3)	0 (0.0)	2 (10.0)	2 (20.0)	3 (15.0)

*One participant had overlap lcSSc and SLE
** All participants positive by multi-bead, ELISA and ID had multi-bead IU values of ≥8.0. No participants negative by multi-bead, ELISA and ID had multi-bead IU values of ≥8.0.

CONCLUSION

- Our results suggest a high rate of false positives for the anti-topo I by multi-bead assay in patients without any clinical evidence of SSc.
- This leads to additional testing, inappropriate referral to rheumatologists, and consternation among patients.
- Limitations: The study only included patients who were positive by multi-bead testing, and tests negative by ELISA were not evaluated further by ID.
- Samples were only sent to a single laboratory (RDL).
- Prevalence of anti-topo I + in SLE was 3.9%, similar to other reports.[4]
- A stepwise approach of confirmation, using both ELISA and ID, greatly improves the predictive value of antibody testing for the diagnosis of SSc.

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