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Advanced Diagnostic Laboratories

Serologic Evaluation in Inflammatory Muscle Diseases and Interstitial Lung Disease: Importance of Autoantibody Testing

Executive Summary

The idiopathic inflammatory myopathies are a group of inflammatory muscle diseases commonly referred to as myositis. This group of autoimmune diseases is characterized by chronic muscle inflammation leading to muscle weakness. Interstitial lung disease (ILD) is often observed and may be the first or only sign of the underlying systemic autoimmune condition. The presence of ILD in myositis patients is typically associated with increased morbidity and mortality.

The presence of myositis-specific autoantibodies in patients can often be indicative of disease [1]. Autoantibody testing has emerged as a useful tool available to physicians evaluating myositis. The Advanced Diagnostic Laboratories at National Jewish Health offer a Myositis Antibody Panel, which assays for myositis-specific and myositis-associated autoantibodies to eleven antigens, including several anti-tRNA synthetase autoantibodies. The panel may be used as an important part of the evaluation in those with myositis or select cases of idiopathic interstitial pneumonia. The eleven autoantibodies detected with the panel are associated with a range of inflammatory muscle diseases. Importantly, this antibody panel is a more comprehensive assay that may help in identifying autoimmune conditions that may not be apparent using single antibody testing alone.

As an example, a recent study from our center [2] described a group of patients presenting with "idiopathic" interstitial pneumonia but who were ultimately diagnosed with anti-synthetase syndrome based on clinical features and anti-PL-7 or anti-PL-12 antibodies. This study demonstrated that patients with presumed idiopathic non-specific interstitial pneumonia should be examined not only for anti-Jo-1 antibodies (the most common anti-synthetase antibody), but also for a fuller range of anti-tRNA synthetase antibodies.

In summary, the Myositis Antibody Panel may be an important part of the evaluation when a patient has select forms of ILD, i.e., non-specific interstitial pneumonia, or when myositis is suspected based on clinical scenario.

The Idiopathic Inflammatory Myopathies, Interstitial Lung Disease, and Autoantibodies

The idiopathic inflammatory myopathies (myositis) include several inflammatory muscle diseases. While typically characterized by chronic muscle inflammation, this group of autoimmune diseases often is associated with interstitial lung disease (ILD), particularly with the anti-synthetase syndrome. Diagnosis of the myopathies can be especially difficult in cases when ILD is the first or only manifestation of the underlying systemic autoimmune disease[3].

In patients with myositis, the presence of myositis-specific autoantibodies is often observed. The autoantibody profile, along with a careful clinical evaluation, can be an important factor in the evaluation. This is especially true of patients who present with ILD, and the clinician must consider causes such as underlying autoimmune disease [4]. However, with the exception of anti-Jo-1 testing, most autoantibodies relevant to myositis are not typically tested in routine diagnosis [5]. Importantly, there is much heterogeneity among different patients in the presentation of anti-synthetase syndrome and associated ILD, and this variability can be associated with various autoantibodies [6], highlighting the need for increased testing of a broader range of autoantibodies in patients with signs of myopathy and/or presumed idiopathic interstitial pneumonia – especially non-specific interstitial pneumonia.

Myositis Antibody Panel offered by Advanced Diagnostic Laboratories

The Advanced Diagnostic Laboratories at National Jewish Health offers a Myositis Antibody Panel. The panel tests for myositis-specific IgG class autoantibodies present to eleven antigens in serum and plasma, as determined by an immunoblot assay [7]. The autoantibodies are assayed using the following panel of antigens:

Myositis-Specific, anti-tRNA synthetase:

Jo-1:	a native Jo-1 antigen (histidyl-tRNA synthetase)
PL-7:	a recombinant PL-7 protein (threonyl-tRNA synthetase)
PL-12:	a recombinant PL-12 protein (alanyl-tRNA synthetase)
EJ:	a recombinant EJ protein (glycyl-tRNA synthetase)
OJ:	a recombinant OJ protein (isoleucyl-tRNA synthetase)

Myositis-Specific, other:

Mi-2:	a recombinant Mi-2 protein
SRP:	a recombinant SRP protein (54kDa, signal recognition particle)
Ro-52:	a recombinant Ro-52 protein (52kDa, myositis-associated antigen)

Myositis-Associated:

Ku:	a recombinant Ku protein
PM-Scl75:	a recombinant 75kDa PM-Scl protein
PM-Scl100:	a recombinant 100kDa PM-Scl protein

These antigens are recognized by both myositis-specific autoantibodies and myositis-associated autoantibodies that may be present in a variety of diseases including polymyositis, dermatomyositis, anti-synthetase syndrome, overlap syndrome, juvenile myositis, interstitial lung disease (ILD), and other connective tissue diseases (such as systemic sclerosis and systemic lupus erythematosus). Results from this qualitative *in vitro* assay are available in 9 days. The immunoblot for myositis was evaluated for clinical concordance in 32 patients from the National Jewish Health Interstitial Lung Disease clinic and 20 normal subjects. Additionally, the performance characteristics of the immunoblot were compared with those of a predicate immunoprecipitation assay. The myositis immunoblot compared favorably with the immunoprecipitation assay, showing 90% or greater concordance for all 11 autoantibodies. Concordance with clinical history was seen in 94% of patient samples.

Table 1** depicts additional descriptions of nine of the eleven myositis-specific antibodies and myositis-associated antibodies along with their clinical associations:

Antibody	Myositis-Specific Abs	Myositis-Associated Ab	Poly-myositis	Dermato-myositis	Anti-Synthetase Syndrome	Overlap Syndrome	Myositis (Juvenile)	Interstitial Lung Disease	Arthritis
Mi-2 *	4-14%	N/A	Rare	Common	N/A	Common	10%	Rare	Occasionally
Ku	N/A	% Unknown	Not Described	Not Described	N/A	Common	Occasionally	Not Described	% Unknown
PM/Scl	N/A	8%	Uncommon	Uncommon	N/A	Common	Occasionally	Rare	Common
Jo-1 *	20%	N/A	Common	Occasion-ally	Marker	Common	Reported	Common	Common
SRP *	4%	N/A	Common	Occasion-ally	N/A	Not Described	Uncommon	Common	Common
PL-7	1-4%	N/A	Occasionally	Common	Marker	Common	Reported	Common	Common
PL-12	1-4%	N/A	Occasionally	Common	Marker	Common	Reported	Common	Common
EJ	1-4%	N/A	Occasionally	Common	Marker	Common	Reported	Common	Common
OJ	1-4%	N/A	Occasionally	Common	Marker	Common	Reported	Common	Common

* Mi-2, SRP and Jo-1 are most common and serve as markers for Myositis.

**Data in table adapted from information provided by RDL Reference Laboratory.

An Under-recognized Cause of Fibrotic ILD—non-anti-JO-1 Anti-Synthetase Syndrome

The anti-synthetase syndrome is a systemic autoimmune disease within the family of poly-/dermatomyositis that is classically characterized by the presence of a circulating tRNA synthetase antibody along with the clinical features of myositis, ILD (usually non-specific interstitial pneumonia), Raynaud's phenomenon, distal digital fissuring ("mechanic hands"), Gottron's sign, and an inflammatory, non-erosive arthropathy. Importantly, many patients with the anti-synthetase syndrome manifest only several of these clinical features, and ILD may be the most clinically apparent manifestation. Although anti-Jo1 antibody is the most commonly identified tRNA synthetase antibody,

the importance of broader autoantibody testing is evidenced by the observation that other (non-Jo1) anti-synthetase antibodies are similarly associated with the syndrome [8, 9].

ILD is a well-known manifestation of the anti-synthetase syndrome and there is a growing body of literature that supports additional anti-synthetase testing beyond anti-Jo1. In a recent study, researchers in the Autoimmune and Interstitial Lung Disease (ILD) Program at National Jewish Health evaluated 37 patients for "idiopathic" interstitial pneumonia with clinical features suggesting anti-synthetase syndrome but who were negative for anti-Jo-1 antibodies [2]. These patients were tested for anti-tRNA synthetase autoantibodies other than anti-JO-1. Nine patients (24%) were found to have non-anti-Jo-1 positive anti-synthetase syndrome based on clinical features and the presence of other anti-tRNA synthetase antibodies (seven patients had anti-PL-7 and two had anti-PL-12 antibodies). These nine patients all had non-specific interstitial pneumonia (i.e., ILD) as the most clinically apparent manifestation of their underlying systemic autoimmune disease. These results indicate that among patients with "idiopathic" interstitial pneumonia – and non-specific interstitial pneumonia in particular – anti-PL-7 and PL-12 antibodies may be more common than previously recognized. The authors suggested that testing for other anti-tRNA synthetase antibodies should be considered in patients with clinical features suggestive of the anti-synthetase syndrome.

Clinical Relevance of the Myositis Antibody Panel

The Myositis Antibody Panel may be a useful tool as part of the comprehensive evaluation of patients with features suggestive of an inflammatory muscle disease (i.e., poly-/dermatomyositis or the anti-synthetase syndrome), select forms of idiopathic interstitial pneumonia (especially non-specific interstitial pneumonia), or other connective tissue diseases.

Clinicians are reminded that autoantibody test results are not intended to be used as the sole means for clinical diagnosis or patient management. False-positives are known to occur with all types of antibody assays – and this Myositis Antibody Panel is no different. A diagnosis of inflammatory muscle disease, anti-synthetase syndrome, or other connective tissue disease is NOT made by autoantibody testing alone. A diagnosis of these scenarios requires a thorough clinical evaluation which is optimized using a multidisciplinary approach.

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