

Enhanced myositis diagnostics with new biomarkers

Newer diagnostic strategies now include autoantibodies as biomarkers to aid phenotype classification, malignancy risk assessment, and therapy prognosis.

By Dr. Jacqueline Gosink

Autoantibodies are important biomarkers in the diagnosis of myositis and are useful for differentiating different subforms of the disease. A broad autoantibody analysis is essential to maximise the serological detection rate. New line blots enable parallel specific detection of up to 20 autoantibodies and thus provide valuable support in the identification of subforms with specific clinical features.

Understanding myositis

Idiopathic inflammatory myopathies (IIM), also known as myositides, are a group of systemic autoimmune rheumatic diseases that are characterised by chronic inflammation of skeletal muscle. They can affect both children and adults. The hallmark symptoms of IIM are muscle inflammation, muscle weakness, arthritis, cutaneous rashes, calcinosis, ulceration, malignancy and interstitial lung disease (ILD).

Based on clinical and immunopathological criteria, the disease is divided into the subforms polymyositis (PM), dermatomyositis (DM), anti-synthetase syndrome (ASS), sporadic inclusion body myositis (sIBM), necrotising myositis (NM) and overlap myositis (OM). OM is the most common form and is characterised by clinical symptoms of IIM, often ASS or DM, together with other autoimmune diseases such as systemic sclerosis, systemic lupus erythematosus, Sjögren's syndrome, or rheumatoid arthritis. Some IIM subforms can be associated with cancer. Large population-based studies have shown a tumour frequency of 20 to 25 per cent in PM/DM, with adenocarcinomas being the most common tumours in myositis patients.

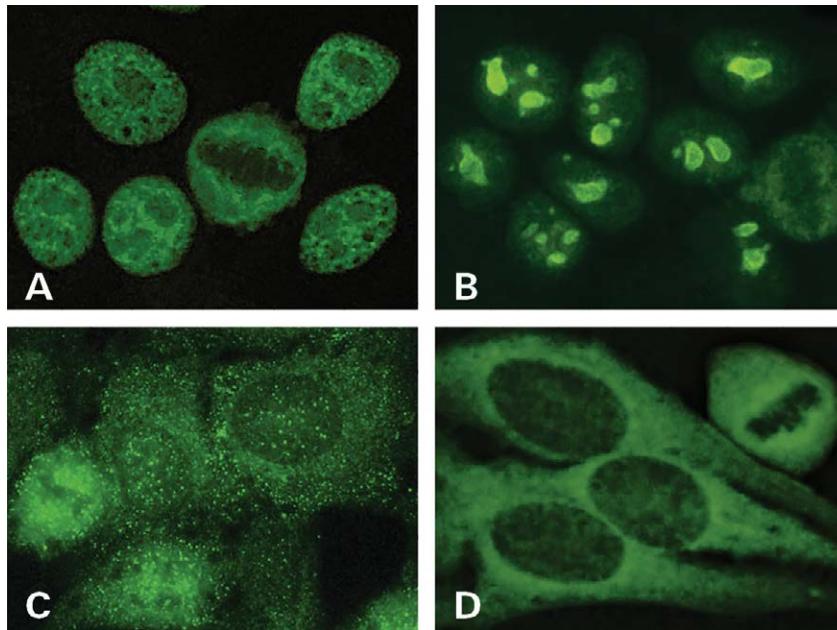


Figure 1. Characteristic immunofluorescence patterns on Hep-2 cells of antibodies against (A) PM-Scl (B) Ku (C) Jo-1 and (D) PL-7.

Diagnosis of myopathies

Diagnosis of myopathies is challenging due to the rarity of the diseases, their similar clinical presentation to other rheumatic diseases, and the possibility of overlap syndromes. The general misdiagnosis rate is high, resulting in a delay to diagnosis of several years. In particular, the rare form sIBM has a mean delay to diagnosis of five to eight years. Accurate diagnosis and differentiation of different IIM subforms is, however, essential due to different therapy regimens. For example, in contrast to PM, sIBM is poorly responsive to immunotherapies.

The current IIM classification criteria published in 2017 by the European League Against Rheumatism (EULAR)/ American College of Rheumatology (ACR) are based predominantly on clinical and

histopathological features. The criteria identify IIM with high sensitivity but may not reliably identify the subform. In recent years the advancement of knowledge and methods to detect myositis-relevant autoantibodies has increased the relevance of these markers in IIM diagnostics.

Newer diagnostic strategies now include autoantibodies as biomarkers to aid phenotype classification, malignancy risk assessment, and therapy prognosis. A plethora of studies have shown that the investigation of autoantibodies alongside clinical evaluation can enhance IIM diagnostics and classification. In Germany, new guidelines published in 2022 by the German Society for Neurology (DGN) now stipulate the determination of autoantibodies as part of the diagnostic procedure.

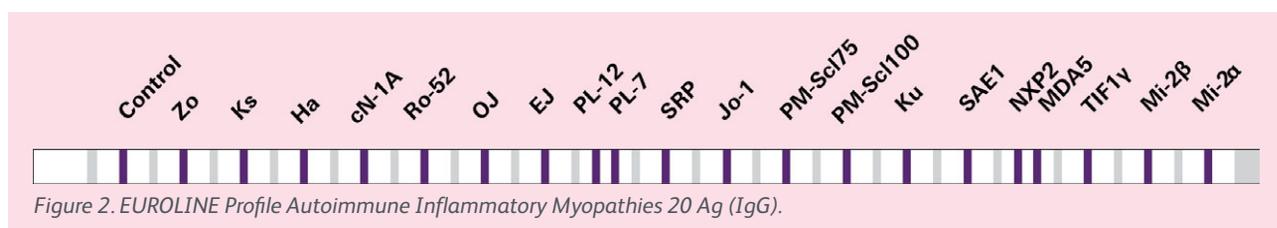


Figure 2. EUROLINE Profile Autoimmune Inflammatory Myopathies 20 Ag (IgG).

MSA against	Associated diseases
cN-1A	sIBM
EJ	ASS, ILD
Ha	ASS
HMGCR	NM
Jo-1	ASS
Ks	ASS
MDA5	DM, ILD
Mi-2 α	DM, caDM
Mi-2 β	DM, caDM
NXP2	DM, ILD, caDM
OJ	ASS, ILD
PL-12	ASS, ILD
PL-7	ASS, ILD
SAE1	DM, ILD, caDM
SRP	ASS, NM
TIF1 γ	DM, caDM
Zo	ASS
MAA against	Associated diseases
Ku	OM
PM-Scl75	OM, DM
PM-Scl100	OM, DM
Ro-52	ILD

Table 1. Autoantibodies and associated disease forms.

Autoantibody markers

Autoantibodies found in IIM are directed against nuclear and cytoplasmic target antigens and are divided into myositis-specific autoantibodies (MSA), which are found primarily in patients with IIM, and myositis-associated autoantibodies (MAA), which are not specific for IIM but are nevertheless important markers. Target antigens of MSA include the nuclear antigens Mi-2 α , Mi-2 β , SAE1, NXP2, MDA5 and TIF1 γ and the cytoplasmic antigens Jo-1, PL-7, PL-12, OJ, EJ, Zo, Ks, H, cN-1A and signal recognition particle (SRP). Target antigens of MAA include the nuclear antigens Ku, PM-Scl75, PM-Scl100 and the cytoplasmic antigen Ro-52.

MSA are rare and typically occur in isolation. Their prevalence ranges from less than one per cent for some anti-tRNA synthetases to 15-20 per cent for anti-Jo1. MAA is detected in up to 50 per cent of myositis patients and additionally in other autoimmune diseases that overlap with myositis. The specificities of myositis autoantibodies are associated with distinct clinical manifestations. Their detection thus provides an indication of the disease subform (Table 1). While several of these autoantibodies have been known since the 1970s and are well characterised, others such as anti-Ks, -Ha, and -Zo are currently being explored regarding their role as biomarkers in IIM and ILD. Further, anti-cN-1A is the first and only known

serological marker for sIBM. Due to its high specificity, it can aid the differentiation of sIBM from other forms of IIM. Detection of anti-cN-1A plays a valuable role in securing an early diagnosis of sIBM and reducing the number of muscle biopsies per person.

Moreover, positivity for certain autoantibodies may provide the first indication of an underlying tumour, enabling the initiation of a comprehensive tumour screening. Autoantibodies against Mi-2 α , Mi-2 β , SAE1, TIF1 γ and NXP2, for example, are associated with an increased risk of cancer in adults.

Autoantibody detection

Autoantibodies in myositis are detected using the indirect immunofluorescence test (IIFT) with human epithelial (HEp-2) cells and primate liver alongside immunoassays such as line blot or ELISA for monospecific characterisation of antibody specificities. IIFT is the gold standard for the detection of anti-nuclear antibodies and many myositis antibodies show a characteristic fluorescence pattern (Figure 1).

However, some autoantibodies, especially cytoplasmic autoantibodies, cannot be clearly identified by IIFT. Therefore, monospecific antibody detection should be performed in parallel. Given the low prevalence and isolated occurrence of many of the autoantibodies in IIM, investigation of a wide range of specific parameters is paramount. Line blots enable many different autoantibodies to be examined in a single test and are thus more informative and efficient than sequential single-parameter tests. Comprehensive studies in various centres in Europe have demonstrated that the simultaneous investigation of myositis antibodies in a large profile can increase the serological detection rate.

Broad-range line blots

The most comprehensive commercially available line blots for myositis diagnostics are EUROLINE Autoimmune Inflammatory Myopathies Profiles, which encompass up to 20 target antigens in one test (Figure 2).

cN-1A is exclusive to the EUROLINE product range and enables the detection

of anti-cN-1A autoantibodies in sIBM. The antigens Ha, Ks and Zo have recently been added to the line blot portfolio, extending the spectrum of tRNA synthetases. Each antigen or group of antigens is printed onto a separate membrane chip to provide optimal efficiency of detection for each antigen. Various profiles are available offering different combinations of myositis-relevant antigens, for example, 20, 17, 16, 11, or 7 antigens per test strip catering to different laboratories' requirements.

Further EUROLINE profiles are also available for autoantibody diagnostics in other rheumatic diseases, for example detection of anti-nuclear antibodies, anti-cytoplasmic antibodies, or antibodies that occur in systemic sclerosis. All EUROLINE assays can be fully automated using the EUROBlotOne device with EUROLineScan software, providing increased standardisation and efficiency.

Outlook

IIM are rare but represent a significant burden for patients. Patients with IIM often experience severe illness due to the damage caused by both the disease and its treatment. In particular, the chronic and slowly progressive nature of sIBM can lead to severe disability. A comprehensive analysis of MSA and MAA in the diagnostic workup can reduce the time to diagnosis, enabling early implementation of targeted therapy. A subset of patients does not, however, exhibit any of the characterised autoantibodies. Therefore, it is likely that further myositis autoantibodies will be discovered in the future.

With line blot technology, new antigenic targets can be easily added to the blot strips to expand the spectrum of testable autoantibodies. Autoantibody markers may also play a more prominent future role as predictors of disease course and therapy outcome. ●

References available on request.

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