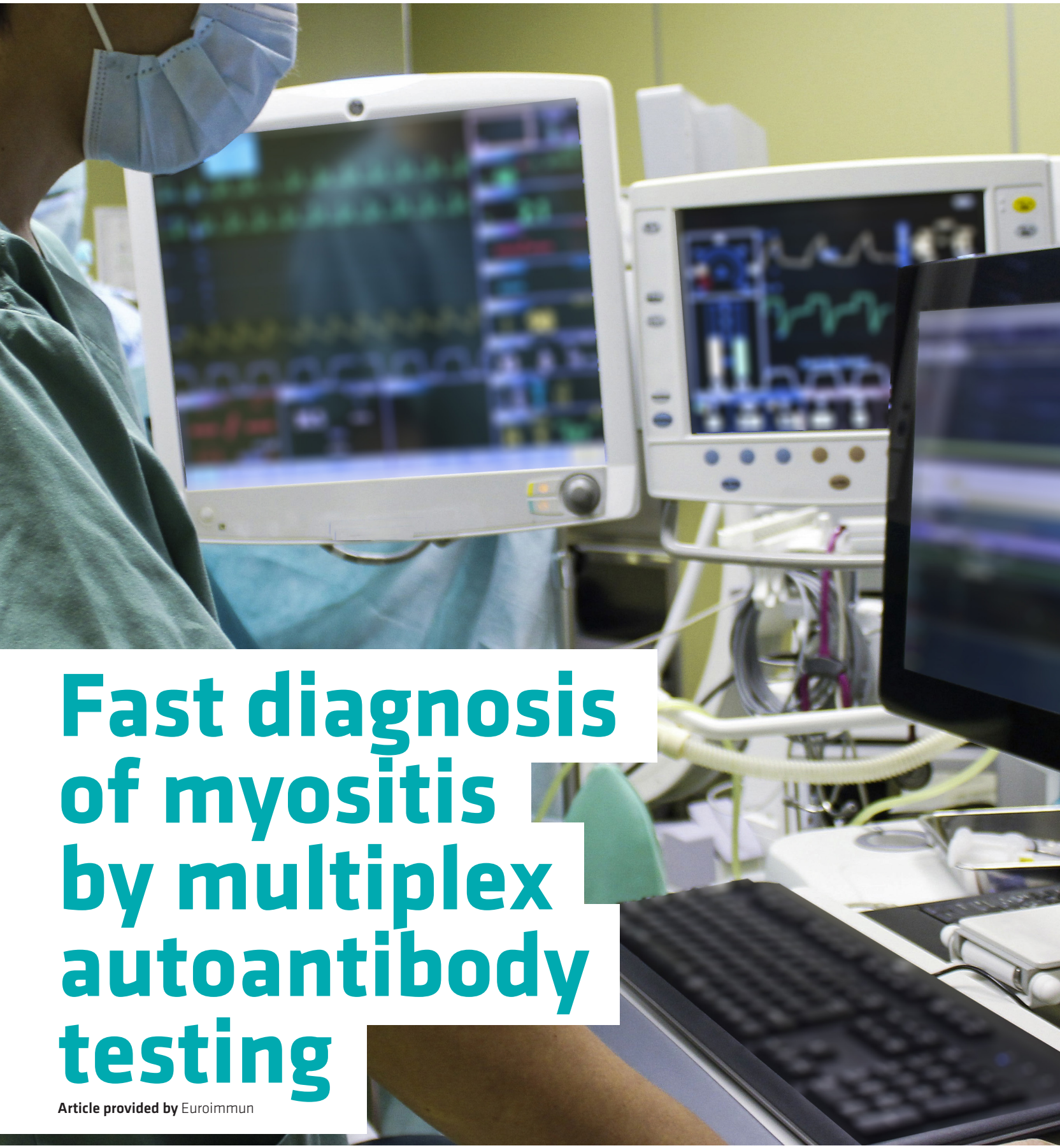


Fast diagnosis of myositis by multiplex autoantibody testing

Article provided by Euroimmun

Autoantibodies are important biomarkers for the diagnosis and classification of idiopathic inflammatory myopathies (IIM). An IIM diagnosis, moreover, necessitates targeted screening for associated underlying tumours. IIM are rare autoimmune diseases of skeletal muscle which can affect both adults and children. The main forms are polymyositis (PM), dermatomyositis (DM) and sporadic inclusion body myositis (sIBM). IIM are characterised by a diverse range of autoantibodies, many of which are themselves rare and typically occur in isolation. Therefore, comprehensive multiparametric testing is essential to maximise the diagnostic information obtained from the analysis.

Polymyositis/dermatomyositis

PM is a systemic inflammatory disease of the skeletal musculature with perivascular lymphocytic infiltration. When the skin is involved, the disease is known as DM. Clinical symptoms of PM are recurring bouts of fever, muscle weakness, arthralgia, Raynaud's syndrome, trouble with swallowing, and involvement of inner organs. In DM, skin symptoms appear as purple-coloured exanthema on the eyelids, nose bridge and cheeks, periorbital oedema, local erythema and scaly eczema dermatitis. PM and DM are associated with a variety of cancers. Large population-based studies have shown a tumour frequency of 20-25%, with a higher occurrence in DM than in PM. The risk, moreover, increases with age. Typical cancers include ovarian, lung, pancreatic, stomach and colorectal tumours in DM and non-Hodgkin lymphoma, lung and bladder tumours in PM.

Autoantibodies in myositis target nuclear and cytoplasmic components of the cell. They 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 the disease but are nevertheless important markers. Target antigens of MSA include the nuclear antigens Mi-2α, Mi-2β, SAE1, NXP2, MDA5 and TIF1γ and the cytoplasmic antigens Jo-1 PL-7, PL-12, EJ, OJ, signal recognition particle (SRP), and further tRNA synthetases. Target antigens of MAA include the nuclear antigens Ku, PM-Scl75, PM-Scl100, and the cytoplasmic antigen Ro-52. The combined frequency of all autoantibodies in myositis amounts to around 60%, whereby there is virtually no overlap in the occurrence of the different antibodies. Comprehensive studies in various centres in Europe have shown that the simultaneous investigation of various myositis-specific antibodies in a large profile significantly increases the serological detection rate.

The autoantibody specificities provide an indication of disease subform (Figure 1), for example, antibodies against Jo-1, PL-7, PL-12, EJ, OJ and SRP are characteristic of PM, while those against Mi-2α, Mi-2β, SAE1, NXP2, MDA5 and TIF1γ occur in DM. Antibodies against PM-Scl75, PM-Scl100 and Ku indicate an overlap syndrome, in particular with the autoimmune connective tissue disease systemic sclerosis. Some antibodies, for example anti-MDA5, -PL7, -PL12, -OJ and -EJ, are associated with an increased risk of interstitial lung disease. Positivity for myositis autoantibodies can also be the first indicator of an underlying tumour. Antibodies against TIF1γ, in particular, have been shown to correlate strongly with malignancies in adults. A myositis diagnosis in adults should always be followed up by tumour screening.

Autoantibody profiling in PM/DM

In suspected cases of PM/DM, patient sera are serologically investigated using indirect immunofluorescence test (IIFT) on a substrate combination of HEp-2 cells and primate liver (Figure 2), with confirmation of results by monospecific tests. Since antibodies against the cytoplasmic antigens are sometimes not clearly detectable with IIFT, parallel performance of the screening and confirmatory test is recommended. Immunoblots are an ideal confirmatory method, as they enable many different antibodies to be monospecifically detected simultaneously. Line blots fitted with individual membrane chips allow antigens with widely differing properties to be combined on one test strip, enabling profiles to be assembled according to the disease application, regardless of the antigens involved.

A unique immunoblot containing 16 antigens focuses exclusively on autoantibodies that occur in autoimmune inflammatory myopathies. The EUROLINE Autoimmune Inflammatory Myopathies 16 Ag (Figure 3) contains the antigens Mi-2α, Mi-2β, TIF1γ, MDA5, NXP2, SAE1, Ku, PM-Scl100, PM-Scl75, Jo-1, SRP, PL-7, PL-12, EJ, OJ and Ro-52 on one test strip.

In four unrelated studies, a total of 804 sera from myositis patients and 786 control sera were investigated with the EUROLINE (Table 1). The antibody prevalences obtained in the myositis patients ranged from 1% for the rare parameters anti-EJ and -OJ to 21% for anti-Jo-1. The specificities for the individual antigens lay between 97-100%. The low prevalences demonstrated for many of the parameters underpins the need for comprehensive multiparametric testing.

Sporadic inclusion body myositis

sIBM is a rare form of IIM. It is a degenerative autoimmune disease of muscle, with inflammatory infiltrates and inclusion vacuoles. Its prevalence is 1 to 71 per million individuals, rising to 139 per million in people over 50 and varying between different populations. Clinical manifestations of sIBM are muscle weakness and atrophy, preferentially affecting the quadriceps femoris and the wrist and finger flexors. The disease is chronic and slowly progressive, leading to severe disability.

sIBM is difficult to distinguish from other IIM. Cases that are suspected on clinical grounds are currently confirmed ▶

▼TABLE 1. Autoantibodies in myositis

Autoantibodies against*	Prevalence	Specificity
Mi-2α	7%	100%
Mi-2β	3-4%	≥98%
MDA5	2%	100%
NXP2	2%	100%
SAE1	4%	100%
Ku	3-5%	≥95%
PM-Scl100	4-7%	100%
PM-Scl75	6%	98%
Jo-1	12-21%	100%
SRP	4%	99%
PL-7/PL-12	2-4%	100%
EJ	1%	100%
OJ	1%	100%
TIF1γ	-	100%

**Analysed in 804 sera from myositis patients and 786 control sera; except for TIF1γ antibodies which were analysed in 10 anti-TIF1γ positive sera and 120 control sera*

by muscle biopsy. However, some of the typical histological features are not detectable in up to 30% of patients. Diagnosis may be assisted by determining autoantibodies against the skeletal muscle antigen cytosolic 5'-nucleotidase 1A (cN-1A, also known as Mup44 or NT5C1A). Anti-cN-1A autoantibodies are currently the only known serum marker for sIBM. Due to their high specificity, their detection can in particular aid the differentiation of sIBM from other muscle diseases such as PM, DM, necrotising myopathy, muscular dystrophy or myasthenia gravis.

Detection of anti-cN-1A

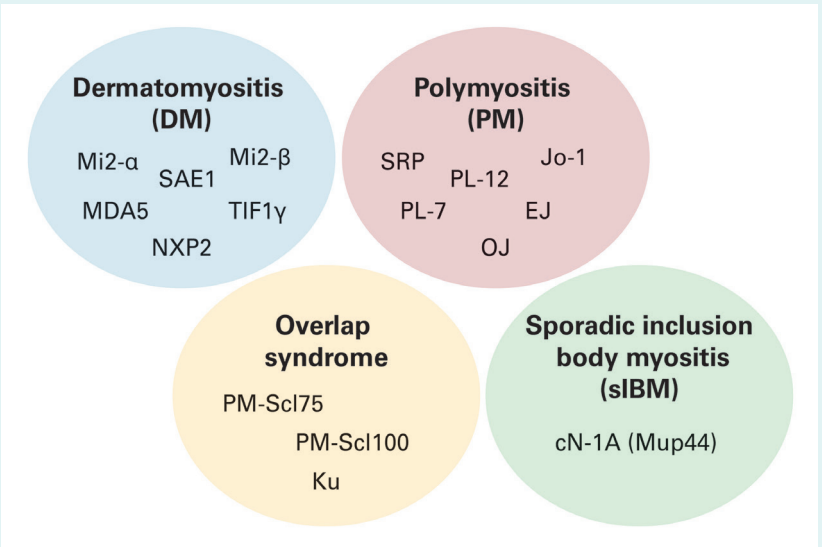
A novel ELISA for the detection of anti-cN-1A autoantibodies has been developed based on recombinant full-length human antigen. Use of the full-length antigen allows detection of antibodies to most, if not all, epitopes. In a recently published study the diagnostic performance of the ELISA was evaluated by two reference laboratories using different serum panels. The first cohort consisted of a total of 286 sera from patients with clinically and pathologically diagnosed definite sIBM, patients with suspected sIBM, myositis controls, non-myositis autoimmune controls and healthy subjects. The second cohort comprised a total of 253 sera from patients with definite sIBM and from healthy controls. Anti-cN-1A reactivity was most frequent in cases of definite sIBM (39% to 47%), but absent in biopsy-proven classic PM or DM. Overall the diagnostic sensitivity amounted to 36% in the first cohort and 39% in the second, while the specificity was 96% or 97%, respectively. Importantly, the sensitivity and specificity measured at the two different laboratories were highly similar. The study thus confirms the high specificity of anti-cN-1A antibodies for sIBM and their utility for differentiating sIBM from other myositides.

Perspectives

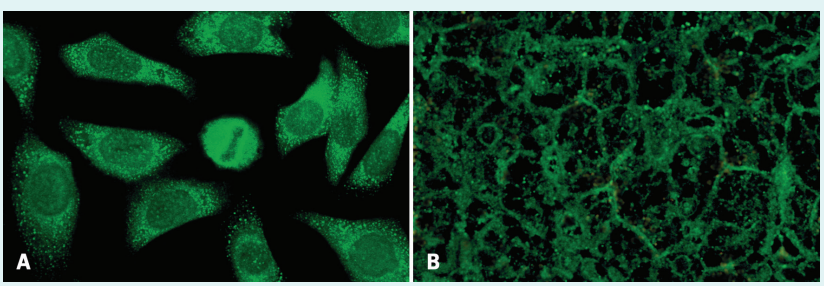
Diagnosis of PM/DM is challenging due to rarity of the diseases, the varying clinical presentation and the possibility of overlap syndromes. The determination of MSA and MAA can significantly reduce the time to diagnosis, with multiparametric testing ensuring the highest serological detection rate. Immunoblots are ideal for multiplex confirmatory testing as they offer broad antigen combinations, easy interpretation and full automatability. The autoimmune inflammatory myopathies profile described here is the most comprehensive line blot for myositis antibodies. It is anticipated that ongoing research will identify further novel autoantibodies in myositis, enabling the diagnostic net to be expanded further. The role of specific myositis autoantibodies as predictors of disease course and therapy responses is also being explored.

The extremely rare form of myositis, sIBM, has a high misdiagnosis rate and a mean delay to diagnosis of 5 to 8 years. Anti-cN-1A testing can play a valuable role in securing an early diagnosis and reducing the number of muscle biopsies per person. Differentiation of sIBM from other idiopathic inflammatory myophathies such as PM is critical due to different treatment regimes. In contrast to PM, sIBM is poorly responsive to immunotherapies. The anti-cN-1A ELISA described here is the first fully standardised commercial assay for routine testing. Further studies are in progress to probe the clinical meaningfulness of anti-cN-1A determination, for example the association of anti-cN-1A antibodies with particular disease features. ML

▼**FIGURE 1:** Characteristic autoantibodies in different myositis subforms



▼**FIGURE 2:** Positive anti-Jo-1 results in IIFT on (A) HEp-2 cells and (B) primate liver



▼**FIGURE 3:** EUROLINE Autoimmune Inflammatory Myopathies 16 Ag Profile