Autoantibodies in myositis: An upgrade for comprehensive serological testing

by Dr Jacqueline Gosink

The autoimmunogenic myositides (idiopathic inflammatory myopathies, IIM) constitute a group of systemic autoimmune rheumatic diseases that are characterized by chronic inflammation of muscles. Unfortunately, the general misdiagnosis rate is high resulting in a delay to diagnosis of several years. Moreover, a significant proportion of patients with IIM suffer from a generalized feeling of severe illness due to the damage caused by both the disease and its treatment. Autoantibodies are useful biomarkers to differentiate clinically indistinguishable subforms of IIM. Despite the low prevalences and isolated occurrence of many autoantibodies in IIM, they constitute the basis for serological diagnostics. The diagnostic information is maximized by employing comprehensive multiparametric assays covering both myositis-specific and myositis-associated autoantibodies.

Myositis

Myopathies are rare, acquired chronic autoimmune diseases of skeletal muscles, which can affect both children and adults. The hallmark symptoms of idiopathic inflammatory myopathies (IIM) are muscle inflammation, proximal muscle weakness and disability, arthritis, cutaneous rashes, calcinosis, ulceration, malignancy and interstitial lung disease (ILD). Both disease and treatment lead to a poorer health status of myositis patients in comparison to the general population [1]. Subforms include polymyositis (PM), dermatomyositis (DM), cancer-related myositis, anti-synthetase syndrome (ASS) and sporadic inclusion body myositis (sIBM).

Subforms of IIM

Polymyositis (PM) manifests with recurring bouts of fever, muscle weakness, arthralgia, Raynaud's syndrome and trouble with swallowing. When the skin is involved, the disease is known as dermatomyositis (DM). In DM, symptoms occur as purple-coloured exanthema on the eyelids, nose bridge and cheeks, periorbital oedema, local erythema and scaly eczema dermatitis. Both PM and DM can be associated with a paraneoplastic syndrome. Large population-based studies have shown a tumour frequency of 20–25% with a higher occurrence in DM than in PM. Adenocarcinomas are the most common tumours in myositis, representing about 70% of malignancies. DM is strongly associated with ovarian, lung, pancreatic, stomach and colorectal cancers and non-Hodgkin lymphoma, whereas PM is associated with lung and bladder cancers and non-Hodgkin lymphoma. In general, the risk of malignancy increases with age.

Among the IIMs, ASS is a severe condition characterized by extramuscular and multiple organ involvement, affecting the lungs in particular. The classical triad of manifestations of ASS are myositis, ILD and non-erosive arthritis, although ASS is characterized by a large heterogeneity in the severity of clinical findings.

Sporadic inclusion body myositis (sIBM) is a rare subform of IIM, which is difficult to distinguish from other subforms. Clinical manifestations of sIBM are dysphagia, 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 has a high misdiagnosis rate and a mean delay to diagnosis of five to eight years. Differentiation of sIBM from other IIM is critical due to different treatment regimes. In contrast to PM, sIBM is poorly responsive to immunotherapies.

Diagnostic relevance of autoantibody profiling in IIM

Several autoantibodies targeting nuclear and cytoplasmic components of the cell are associated with IIM. The autoantibodies are divided into myositis-specific autoantibodies (MSA), which are found primarily in patients with IIM, and myositis-associated autoanti-

bodies (MAA), which are unspecific for IIM and occur also in other diseases, but are nevertheless important diagnostic markers [2]. The isolated presence of MSA against an individual antigen is characteristic for autoimmune myositis. The autoantibody specificity correlates with pathogenesis and is associated with distinct clinical manifestations, thus providing an indication of the disease subform as shown in Table 1 [3]. 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, KS, Zo, Ha-YRS and signal recognition particle (SRP). Target antigens of MAA include the nuclear antigens Ku, PM-Scl75, PM-Scl100 and the cytoplasmic antigen Ro-52. The prevalences of the autoantibodies in IIM range from 1% for the rare parameters anti-EJ and anti-OJ antibodies to around 20% for anti-Jo-1 antibodies.

Myositis-specific autoantibody	Associated subform of IIM
cN-1A	sIBM
EJ	ASS, ILD
Ha-YRS	ASS
HMGCR	NM
Jo-1	ASS
KS	ASS
MDA5	DM, ILD
Mi-2α	DM, caDM
Mi-2β	DM, caDM
NXP2	DM, ILD, caDM
OJ	ASS, ILD
PL-12	ASS, ILD
PL-7	ASS, ILD
SAE1	DM, ILD, caDM
SRP	ASS, NM, cardiac involvement
TIF1γ	DM, caDM
Myositis-associated autoantibody	**Associated subform of IIM**
Ku	OS
PM-Scl75	OS, DM
PM-Scl100	OS, DM
Ro-52	ILD

Table 1. Myositis-specific and associated autoantibodies and the corresponding subform of IIM ASS, anti-synthetase syndrome; caDM, cancer-associated dermatomyositis; DM, dermatomyositis; ILD, interstitial lung disease; NM, necrotizing myopathy; OS, overlap syndromes; sIBM, sporadic inclusion body myositis.

Newer diagnostic strategies now include MSA as biomarkers to aid phenotype classification, malignancy risk assessment and therapy prognosis [4]. Autoantibodies against the aminoacyl tRNA synthetases Jo-1, PL-7, PL-12, EJ, OJ, KS, Zo and Ha-YRS are characteristic of ASS, while those against Mi-2α, Mi-2β, SAE1, NXP2, MDA5 and TIF1γ occur in DM. Autoantibodies against PM-Scl75, PM-Scl100 and Ku indicate an overlap syndrome, in particular with the autoimmune connective tissue disease systemic sclerosis. The anti-Ro-52, -MDA5, -PL-7, -PL-12, -OJ, and -EJ autoantibodies are associated with an elevated risk of ILD. Positivity for myositis autoantibodies can be the first indicator of an underlying tumour. In particular, autoantibodies against SAE1, TIF1γ and NXP2 are associated with an increased risk of cancer in adult patients with IIM. Also the production of anti-Jo1 and anti-PL-12 autoantibodies may be driven by malignancy. Consequently, a myositis diagnosis in adults should always be followed up by tumour screening.

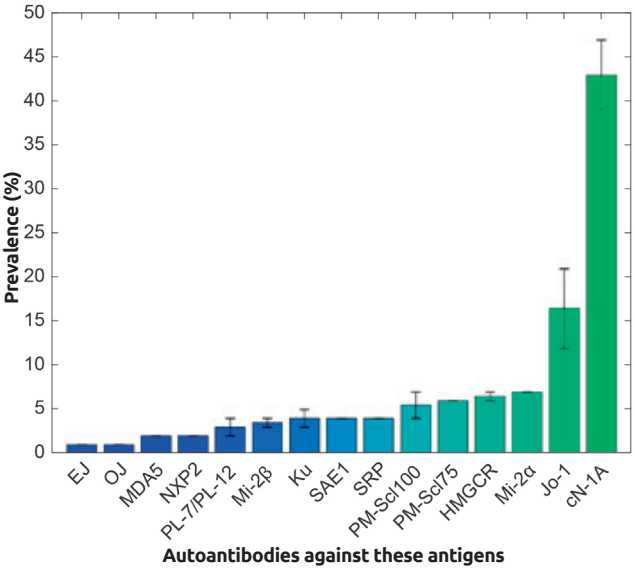

Figure 1. Prevalence of autoantibodies in myositis [3, 5–7, 9–11] Error bars represent the range of prevalence. Note that the prevalence of anti-cN-1A antibodies refers to sIBM patients.

Comprehensive studies in various centres in Europe have shown that the simultaneous investigation of myositis-specific antibodies in a large profile can significantly increase the serological detection rate [3, 5, 6]. The specificities for the individual antigens were between 97% and 100%. Importantly, the autoantibody prevalences were low, amounting to under 10% for most autoantibodies (exception: anti-Jo-1 with a prevalence ranging between 12% and 21%) (Fig. 1). These low prevalences underpin the need for comprehensive multiparameter testing in suspected cases to maximize the autoantibody detection rate allowing accurate serodiagnosis.

New autoantibodies in IIM: anti-cN1A, anti-HMGCR, anti-Ks, anti-Ha, anti-Zoα autoantibodies

While several MSA and MAA are known since the mid-1970s, new candidates are currently explored regarding their potential role as biomarkers for IIM.

Autoantibodies against the skeletal muscle antigen cytosolic 5'-nucleotidase 1A (cN-1A) are the only currently known biomarker 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, necrotizing myopathy (NM), muscular dystrophy or myasthenia gravis. The detection of anti-cN-1A autoantibodies may facilitate early diagnosis of sIBM, especially when the clinical picture is unclear and/or when typical pathological features are not yet detectable. In sIBM, the detection of anti-cN-1A antibodies can play a valuable role in securing an early diagnosis and reducing the number of muscle biopsies per person. Two reference laboratories used two different serum panels to evaluate the diagnostic performance of the Anti-cN-1A ELISA (EUROIMMUN) (Fig. 1) [7]. The study found a prevalence of anti-cN1A autoantibodies in sIBM to be 39–47% and confirmed both the high specificity of anti-cN-1A autoantibodies for sIBM and their utility for differentiating sIBM from other IIMs. Further studies are in progress to probe the clinical meaningfulness of anti-cN-1A antibody determination, for example the association of anti-cN-1A antibodies with particular disease features [8].

Specific autoantibodies against 3-hydroxy-3-methylglutaryl coenzyme A reductase (anti-HMGCR) are detected in 6–7% of cases of IIM (Fig. 1) [9–11]. A small number of patients who take statins develop NM, which is associated with anti-HMGCR antibodies in up to 75% of these patients [9]. The antibody titre correlates with the clinical activity of NM. Nevertheless, NM with anti-HMGCR antibodies may also occur without the intake of statins.

In a recent exploratory study, patients with ILD and healthy subjects were tested for the presence of autoantibodies targeting asparaginyl-transfer-RNA synthethase (anti-Ks), tyrosyl transfer-RNA synthethase (anti-Ha), phenylanyl-transfer-RNA synthethase alpha (anti-Zoα) [12]. Autoantibodies were detected in serum using a line blot assay (EUROLINE Myositis Research Profile, EUROIMMUN). The prevalences of antibodies specific for Ks, Ha and Zoα in ILD were 1.3%, 2.0% and 1.4%, respectively. Anti-Ha and anti-Ks autoantibodies were observed in males with unclassifiable idiopathic interstitial pneumonia, hypersensitivity pneumonitis, and various connective tissue diseases (CTD-ILD), which are an important secondary cause of ILD. Anti-Zoα autoantibodies were associated with CTD-ILD. It is as yet undefined whether these autoantibodies will be added to the standard set of autoantibodies that is conventionally analysed for laboratory diagnostics of IIM.

Serological testing strategies in IIM

To serologically investigate samples from patients with suspected IIM, indirect immunofluorescence tests (IIFT) and immunoblots, as monospecific confirmatory tests, are employed. Due to its high sensitivity and specificity, the IIFT with human epithelial cells and primate liver is the gold standard screening test for the detection of anti-nuclear antibodies. Because antibodies against the cytoplasmic antigens are sometimes not clearly detectable with IIFT, a confirmatory test is essential. Immunoblots enable monospecific but simultaneous detection of many different antibodies. In particular, 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. The line blot is valid, specific and useful to identify subgroups of IIM with specific clinical features and in accordance with the European League Against Rheumatism [10]. To achieve a high serological detection rate, rare autoantibodies with a low prevalence should be included in the testing. The EUROLINE Autoimmune Inflammatory Myopathies Profile (EUROIMMUN) provides up to eighteen MSA including cN-1A and HMGCR on one test strip, enabling most comprehensive analysis of autoantibodies in myositis.

Conclusions and outlook

Diagnosis of PM and DM is challenging owing to the rarity of the diseases, their similar clinical presentation and the possibility of overlap syndromes. An extensive analysis of both MSA and MAA biomarker can reduce the time to diagnosis, ensure the highest serological hit rate and permits better categorization of patients.

Immunoblots are an ideal tool for multiparametric confirmatory testing, as they offer broad antigen combinations, easy interpretation and full automatability. The autoimmune inflammatory myopathies immunoblot described here is the most comprehensive commercially available line blot for IIM antibodies, and will soon be expanded further with the recently identified synthetase target antigens KS, Ha and Zoα.

Since a subset of myositis patients exhibit none of the so far characterized IIM autoantibodies, further antigenic targets will be likely identified in the future. The role of autoantibodies in the pathogenesis of myositis and as predictors of the disease course and treatment outcome will be further explored.

The author

Jacqueline Gosink PhD

EUROIMMUN Medizinische Labordiagnostika AG, 23560 Lübeck, Germany

E-mail address: j.gosink@euroimmun.de

References

1. Sultan SM, Ioannou Y, Moss K, Isenberg DA. Outcome in patients with idiopathic inflammatory myositis: morbidity and mortality. Rheumatology 2002; 41(1): 22–26.
2. Merlo G, Clapasson A, Cozzani E, *et al.* Specific autoantibodies in dermatomyositis: a helpful tool to classify different clinical subsets. Arch Dermatol Res 2017; 309(2): 87–95.
3. Gunawardena H, Betteridge ZE, McHugh NJ. Newly identified autoantibodies: relationship to idiopathic inflammatory myopathy subsets and pathogenesis. Curr Opin Rheumatol 2008; 20(6): 675–680.
4. McGrath ER, Doughty CT, Amato AA. Autoimmune myopathies: updates on evaluation and treatment. Neurotherapeutics 2018; 15(4): 976–994 (https: //doi.org/10.1007/s13311-018-00676-2).
5. Targoff IN. Myositis specific autoantibodies. Curr Rheumatol Rep 2006; 8(3): 196–203.
6. Rönnelid J, Barbasso S, Storfors H, *et al.* Use of a commercial line blot assay as a screening test for autoantibodies in inflammatory myopathies. Autoimmun Rev 2009; 9(1): 58–61.
7. Kramp SL, Karayev D, Shen G, *et al.* Development and evaluation of a standardized ELISA for the determination of autoantibodies against cN-1A (Mup44 , NT5C1A) in sporadic inclusion body myositis. Autoimmun Highlights 2016; 7(16): 1–9.
8. Mende M, Borchardt-Lohölter V, Meyer W, *et al.* Autoantibodies in Myositis. How to achieve a comprehensive strategy for serological testing. Mediterr J Rheumatol 2019; 30(3): 155–161.
9. Mohassel P, Mammen AL. Anti-HMGCR myopathy. J Neuromuscul Dis 2018; 5(1): 11–20.
10. Mammen AL. Statin-associated autoimmune myopathy. N Engl J Med 2016; 374: 664–669.
11. Padala S, Thompson PD. Statins as a possible cause of inflammatory and necrotizing myopathies. Atherosclerosis 2012; 222(1): 15–21.
12. Moll SA, Platenburg MGJP, Platteel ACM, *et al.* Prevalence of novel myositis autoantibodies in a large cohort of patients with interstitial lung disease. J Clin Med 2020; 9(9): 2944.