Review

Journal of INTERNAL MEDICINE

doi: 10.1111/joim.12451

Myositis-specific autoantibodies: an important tool to support diagnosis of myositis

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Abstract. Betteridge Z, McHugh N (University of Bath; and Royal National Hospital for Rheumatic Disease, Bath, UK). Myositis-specific autoantibodies: an important tool to support diagnosis of myositis (Review Symposium). *J Intern Med* 2016; **280**: 8–23.

The idiopathic inflammatory myopathies are characterized by muscle weakness, skin disease and internal organ involvement. Autoimmunity is known to have a role in myositis pathogenesis, and myositis-specific autoantibodies, targeting important intracellular proteins, are regarded as key biomarkers aiding in the diagnosis of patients. In recent years, a number of novel myositis autoantibodies including anti-TIF1, anti-NXP2, anti-MDA5, anti-SAE, anti-HMGCR and anti-cN1A have been identified in both adult and juvenile patients. These autoantibodies correlate with distinct clinical manifestations and importantly are found in inclusion body, statin-induced, clinically amyopathic and juvenile groups of myositis patients, previously believed to be mainly autoantibody negative. In this review, we will describe the main myositis-specific and myositis-associated autoantibodies and their frequencies and clinical associations across different ages and ethnic groups. We will also discuss preliminary studies investigating correlations between specific myositis autoantibody titres and clinical markers of disease course, collectively demonstrating the utility of myositis autoantibodies as both diagnostic and prognostic markers of disease.

Keywords: Autoantibodies, Autoimmunity, Dermatomyositis, Myositis, Polymyositis.

Introduction

The idiopathic inflammatory myopathies (IIMs) are rare autoimmune diseases affecting both adults and children. The conditions are hallmarked by muscle inflammation, leading to proximal muscle weakness and disability, distinct cutaneous rashes, ulceration, calcinosis, malignancy and interstitial lung disease (ILD). Autoimmunity is believed to have a key role in the pathogenesis of myositis and, as such, autoantibodies have been identified in over 50% of patients with IIM. It has been demonstrated that these autoantibodies target both nuclear and cytoplasmic components of the cell and they have traditionally been divided into two subsets, myositis-associated autoantibodies (MAAs) and myositis-specific autoantibodies (MSAs).

Whilst diagnostic and classification criteria for myositis may include a range of diagnostic tests such as electromyography (EMG), muscle biopsy and muscle enzymes, the inclusion of MSAs/MAAs is often limited. This is due to the fact that most of the criteria were established prior to the identification of a significant proportion of the MSA/MAA repertoire, as well as a limited availability of commercial diagnostic testing [1]. However, because the MSAs/MAAs have been extensively demonstrated to correlate with specific clinical manifestations, it is clear that they are important biomarkers for myositis, aiding in diagnosis and helping to classify patients into more homogeneous groups (Table 1). Myositis autoantibodies may therefore aid in predicting additional clinical complications and response to treatment. In this review, we highlight the key MAAs and MSAs and their clinical associations in both adult and juvenile myositis patients.

Methods of autoantibody detection

Myositis-specific autoantibodies/MAAs are routinely detected by a variety of methods, with each assay having distinct advantages and disadvantages of sensitivity, specificity, throughput, cost and required expertise [2, 3] (Fig. 1). For general

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Table 1 *Clinical associations and frequency of myositis-specific autoantibodies*

	Autoantigen	Frequency	Myositis	Rash	Respiratory	Cancer	Necrotizing myopathy	Other
ASA	Aminoacyl tRNA synthetases	Jo-1: 9–24% adult PM/DM Non-Jo-1: <5% adult PM/DM 2–4% JDM	Jo-1 > non-Jo-1	Non-Jo-1 > Jo-1	ILD: non-Jo-1 > Jo-1			Mechanics hands Arthritis Raynaud's phenomenon
SRP	Signal recognition particle	5% Caucasian adults PM/DM 8–13% Asian/African adult PM/DM <2% JDM	Highly elevated CK Severe weakness				Significantly associated	Dysphagia Cardiac involvement? Arthritis?
HMGCR	3-hydroxy-3-methylglutaryl-coenzyme A reductase	6% adult PM/DM <1% JDM	Highly elevated CK				Significantly associated	Statins
Mi-2	Nucleosome-remodelling deacetyalse complex	9–24% adult IIM 4–10% JDM		Significantly associated				
SAE	Small ubiquitin-like modifier activating enzyme	6–8% Caucasian adult PM/DM 2% Asian adult PM/DM <1% JDM	Amyopathic at onset	Significantly associated				Dysphagia?
MDA5	Melanoma differentiation-associated gene 5	10–48% Asian adult DM 0–13% Caucasian adult DM 7–38% JDM	Amyopathic	Significantly associated	ILD RP-ILD in Asian cohorts			
NXP2	Nuclear matrix protein 2	1–17% adult PM/DM 23–25% JDM		Significantly associated		Possible association in adults		Calcinosis in juveniles

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Table 1 (*Continued*)

	Autoantigen	Frequency	Myositis	Rash	Respiratory	Cancer	Necrotizing myopathy	Other
TIF1	Transcriptional intermediary factor 1	13–31% adult PM/DM 22–29% JDM		Significantly associated		Strongly associated in adults		
cN1A	Cytosolic 5'nucleotidase 1A	0–5% adult PM/DM 33–34% IBM	IBM					

IIM, idiopathic inflammatory myopathies; DM, dermatomyositis; PM, polymyositis; JDM, juvenile dermatomyositis; CK, creatine kinase; ILD, interstitial lung disease; RP-ILD, rapidly progressive interstitial lung disease.

diagnostic laboratories, standard tests include indirect immunofluorescence using HEp2 cells, gel-based techniques of counter-immune electrophoresis and immunodiffusion, and enzyme-linked immunosorbent assays (ELISAs) [4].

Immunofluorescence is one of the most commonly used methods for testing autoantibodies; however, the assay requires specialist skills to review the patterns and, in the case of myositis autoantibodies, the results are often negative or nonspecific. Therefore, although immunofluorescence is useful as a validation of myositis autoantibody results acquired by other assays, it is not sufficient to detect all MSAs/MAAs alone [2, 5, 6]. The gel precipitation assays (counter-immune electrophoresis and immunodiffusion) have also been used for MSA/MAA detection, and although the use of a generic antigen source enables screening of several MSAs/MAAs, the assays have a low sensitivity and cannot detect all myositis autoantibodies [3]. For this reason, these assays have been largely superseded by ELISAs using either a generic or a specific antigen source [3, 7]. These assays have the advantage of being high-throughput techniques, with the ability to screen multiple serum samples simultaneously, and can provide quantitative titre information [3]. However, the binding of antigens to a plastic plate can result in the loss of some conformational epitopes [2], and currently, only a limited number of MSAs/MAAs can be detected by the commercially available ELISAs [7, 8].

In addition to the routine assays, specialist laboratories are also able to detect a wider range of the MSAs/MAAs using immunoblotting and line-blotting, radiolabelled immunoprecipitation (IPP) and a number of novel commercial multiplex assays [2, 4]. IPP has been regarded as the gold standard testing method for autoantibody serology due its high sensitivity and ability to detect a wide repertoire of known and unknown autoantigen targets [4, 5]; however, the test requires a specialist centre and is both labour-intensive and time-consuming. IPP also has the disadvantage of not being able to distinguish between autoantibodies targeting proteins of the same molecular weight [3, 9]. Traditional immunoblots using a generic antigen source have similar disadvantages to IPP; however, the development of recombinant line-blots has led to easy-to-use semiquantitative assays. These commercial assays screen for the more prevalent MSAs/

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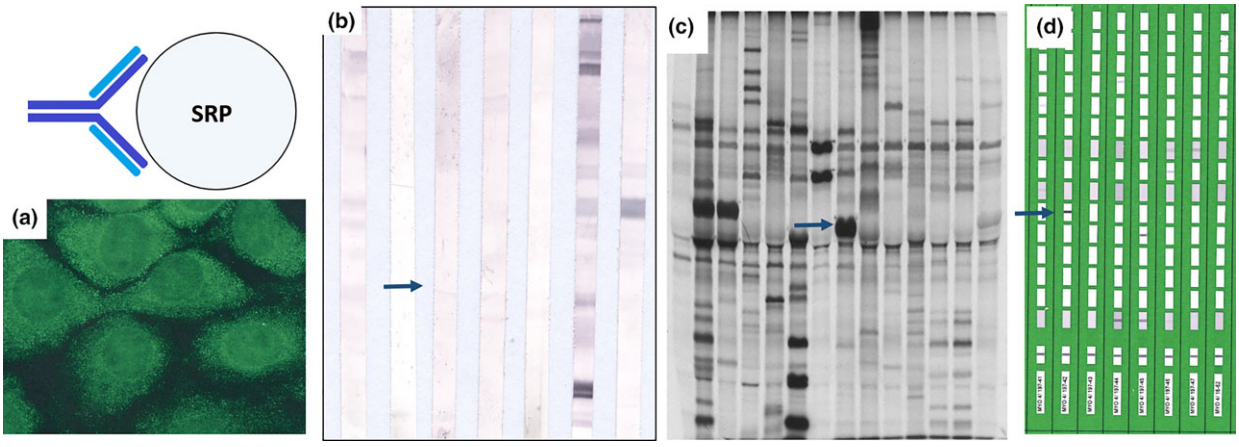

Fig. 1 *Example of laboratory methods used to detect anti-SRP autoantibodies in a single sample. (a) Immunofluorescence on HEp2 cells. The sample results in a fine cytoplasmic speckle; however, although the pattern corresponds with anti-SRP, a fine cytoplasmic speckle is not exclusive for anti-SRP. (b) Immunoblot using K562 cell extracts as a generic antigen source. The signal recognition particle (SRP) antigen is marked; however, as seen from the faint line, the method has a low sensitivity for some myositis-specific autoantibodies (MSAs). (c) Immunoprecipitation using radiolabelled K562 cell extracts; the SRP antigen is marked with an arrow. The method is more sensitive than immunoblot, but is time-consuming and requires a specialist centre. (d) Commercial assays are increasing available (example shown here is a EuroImmun Line Blot, with the anti-SRP antigen marked with an arrow); the commercial assays are generally high-throughput and easy to use, but many still require full validation, and the use of recombinant proteins as the antigen source restricts the ability to detect rare or novel MSAs.*

MAAs, and whilst they have been validated against in-house assays, they remain less sensitive for some of the autoantigen targets than other tests [10].

Finally, due to the substantial progress made in identifying novel myositis autoantibodies in the last decade, a number of commercial companies have produced multiplex assays for the detection of MSAs/MAAs. Although most of these systems still need to be fully validated, it is likely that these newer systems will be become the benchmark for diagnostic myositis autoantibody testing in the future [2, 7].

Frequencies and clinical associations of the myositis autoantibodies

MAAs

Collectively, the MAAs form the largest group of myositis autoantibodies and are found in approximately 20% of adult patients. Although they are less specific for myositis, and are often found in other connective tissue diseases (CTDs), they are still an important diagnostic marker and can correlate with clinical features. One of the most commonly occurring MAAs is anti-PMScl, which targets the 75-kDa and 100-kDa subunits of the nucleolar exosome complex and is reported to occur in 4–12% of patients [11]. Anti-PMScl autoantibodies are found in patients of all ethnic groups and ages, although they are less common in Asian populations (0–2%) [12, 13] and juvenile cohorts (~4%) [14]. Although anti-PMScl autoantibodies have been identified in various CTDs [13], they are most commonly associated with polymyositis (PM) and scleroderma overlap, conferring an increased risk of ILD, inflammatory joint disease, mechanics hands and Raynaud's phenomenon. Preliminary work by Plestilova *et al.* has demonstrated that anti-PMScl-100 levels correlate with serum creatine kinase (CK) activity in dermatomyositis patients with DM and constitutional disease activity and dysphagia severity in patients with PM. In the same study, serial anti-PMScl levels were correlated with HAQ disability scores across all patients with myositis and global disease activity scores in the DM group [15]. Anti-PMScl autoantibodies were originally reported to be a marker of good prognostic outcome; however,

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although improvement was observed in 70% of cases, in a recent study of long-term outcome of anti-PMScl patients with PM/DM, only 10% of the patients achieved remission and 20% had worsened clinical status [16].

A further subset of myositis overlap patients comprises patients with anti-U1-snRNP autoantibodies. The U1-small nuclear ribonucleic proteins (snRNPs) are involved in pre-messenger RNA processing and collectively are composed of at least 11 polypeptides and five snRNP molecules. Although anti-U1-snRNP autoantibodies are only found in 3–8% of adult and juvenile patients with PM or DM, they are much more common in patients with overlap conditions (25–40%) and are generally found in patients with myositis and mixed CTD overlap. Such patients rarely have myositis at initial presentation and have been reported to respond favourably to steroid treatment, suggesting that anti-U1-snRNP autoantibodies are a marker of good prognosis in myositis [11, 14, 17].

Autoantibodies to the 70- and 80-kDa Ku heterodimers are also found in 1–3% of PM and DM patients. Anti-Ku autoantibodies were originally reported in patients with myositis/scleroderma overlap; however, subsequent studies have shown that these autoantibodies occur in a range of CTD conditions and are found in 9–19% of myositis patients with overlap syndromes including systemic lupus erythematosus (SLE), scleroderma and mixed and undifferentiated CTD [11]. Patients with anti-Ku autoantibodies have been reported to have an increased frequency of arthralgia, Raynaud's phenomenon and musculoskeletal manifestations, with myositis anti-Ku-positive patients also having a high frequency of ILD. Anti-Ku-positive myositis patients have been reported to require high-dose corticosteroids, and whereas their musculoskeletal manifestations appear to respond well to treatment, ILD manifestations are severely corticosteroid resistant [18].

Finally, autoantibodies to SSA (Ro60, Ro52) and SSB (La) are also commonly reported in myositis patients. Anti-SSA occurs in 9–19% of adult PM/DM patients, approximately 6% of juvenile DM (JDM) patients and 14–25% of myositis overlap patients, and anti-SSB occurs in 2–7% and 4–12% of PM/DM and overlap patients, respectively [11]. Clinically, SSA has been associated with heart disease in neonatal lupus; however, no specific significant associations have been described in PM/DM for SSA or SSB. Autoantibodies targeting Ro52 have also been reported in a range of autoimmune disorders, and whereas anti-Ro52 autoantibodies have been shown to occur at a similar frequency to anti-Ro60 in most CTDs, anti-Ro52 is far more common than anti-Ro60 in myositis, being described in over 30% of patients [19]. Anti-Ro52 autoantibodies occur frequently with the antisynthetase autoantibodies (ASAs), with studies demonstrating the presence of anti-Ro52 in 56–72% of anti-Jo-1-positive patients. Patients with both anti-Jo-1 and anti-Ro-52 autoantibodies have an increased risk of mechanics hands and malignancy and a poorer outcome than patients with anti-Jo-1 alone. Furthermore, patients with anti-Jo-1 and anti-Ro-52 autoantibodies have a decreased functional status at long-term follow-up and a poorer prognosis than patients with anti-Jo-1 autoantibodies alone [20]. These data therefore demonstrate a diagnostic utility for testing anti-Ro52 alongside the MSAs.

MSAs

Over recent years, an increasing number of MSAs have been identified and characterized, with MSAs collectively now being found in approximately 50% of PM and DM patients. The MSAs are predominately specific for myositis and, with a few rare exceptions, generally mutually exclusive. Numerous studies have demonstrated that each MSA correlates with a distinct clinical phenotype, making MSAs important diagnostic biomarkers (Fig. 2).

Antisynthetase autoantibodies

The most prevalent MSA in myositis is anti-Jo-1, occurring in 9–24% of adult patients with IIM. This autoantibody targets histidyl tRNA synthetase, one of the aminoacyl tRNA synthetases. The tRNA synthetases are a family of cytoplasmic enzymes responsible for the loading of specific amino acids to their cognate tRNA to form an aminoacyl tRNA. To date, a total of eight anti-tRNA synthetase autoantibodies (ASAs) have been reported in myositis, with the additional ASAs, anti-PL12, anti-PL7, anti-EJ, anti-OJ, anti-KS, anti-Zo and anti-Ha, being less common, each occurring in up to 5% of myositis patients and collectively occurring in approximately 6–12% of patients [11, 21]. ASAs have been reported in juvenile myositis patients, but at a much reduced frequency, with

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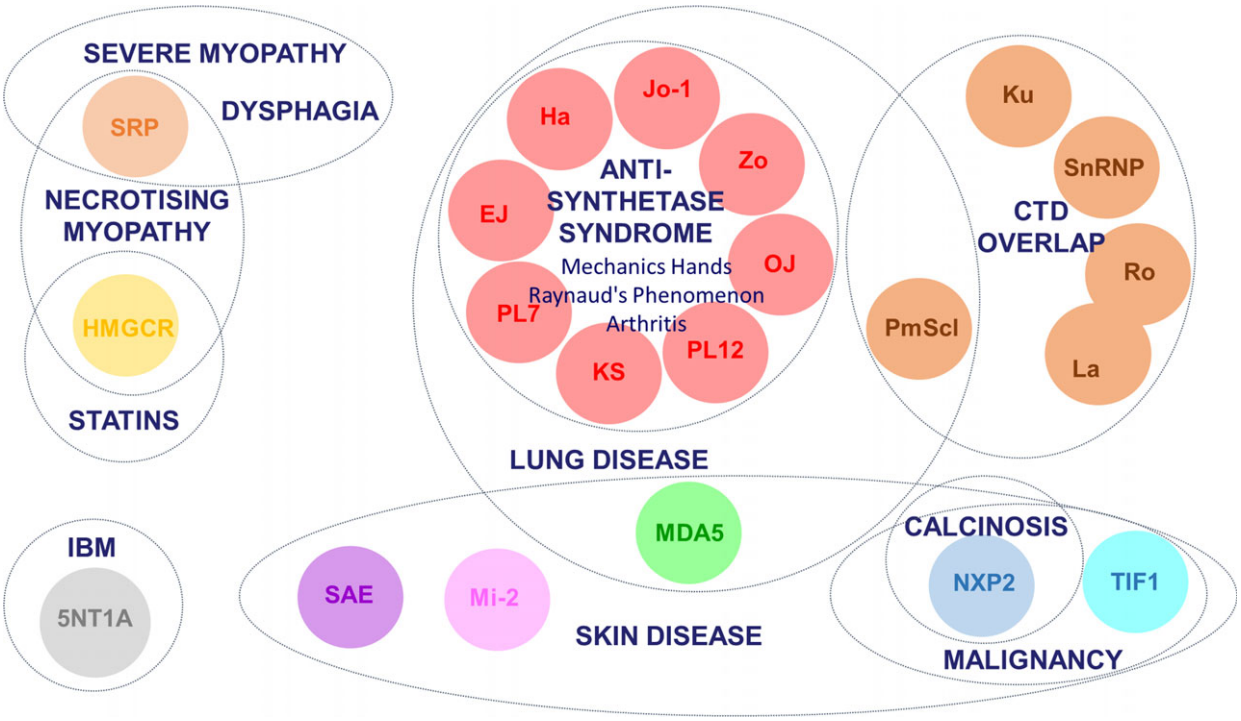

Fig. 2 *Myositis autoantibodies and their key clinical associations. IBM, inclusion body myositis; CTD, connective tissue disease; SRP, signal recognition particle; HMGCR, 3-hydroxy-3-methylglutaryl-coenzyme A reductase; TIF1, transcription intermediary factor 1; NXP2, nuclear matrix protein 2; MDA5, melanoma differentiation-associated gene 5; SAE, small ubiquitin-like modifier activating enzyme; 5NT1A, cytosolic 5'nucleotidase 1A; Mi-2, nucleosome-remodelling deacetyalse complex; Jo-1, histidyl tRNA synthetase; PL7, threonyl tRNA synthetase; PL12, alanyl tRNA synthetase; OJ, isoleucyl tRNA synthetases; EJ, glycyl tRNA synthetase; KS, asparaginyl tRNA sythetase; Zo, phenylalanyl tRNA synthetase, Ha; tyrosyl tRNA synthetase; snRNP, small nuclear ribonucleic protein.*

two large juvenile cohort studies only identifying ASAs in 2–4% of their patients [14] (S Tansley, personal communication).

Patients with ASAs have been traditionally classed as having antisynthetase syndrome (ASS), the clinical manifestations of which are ILD, Raynaud's phenomenon, mechanics hands, nonerosive arthritis, fever and, in some cases, cutaneous rash. Although all the ASAs are associated with ASS, recent studies have demonstrated that the precise clinical manifestations associated with each individual autoantibody are not identical. A meta-analysis comparing anti-Jo-1-positive and non-Jo-1 ASA-positive patients reported an increased likelihood of myositis, arthralgia and mechanics hands in anti-Jo-1-positive patients, with non-Jo-1 ASA-positive patients having an increased frequency of DM skin lesions, fever and ILD [11]. Furthermore, anti-Jo-1-positive patients have been shown to have a better 5- and 10-year survival compared to non-Jo-1 ASA-positive patients, although this may be partially explained by a greater availability of anti-Jo-1 testing compared to the other ASAs, and a subsequent reduced time to diagnosis [22]. Recently, a study of 166 Japanese ASA-positive patients has revealed further clinical differences between the non-Jo-1 subtypes. Muscle weakness was found to be more pronounced at both disease onset and during follow-up in the anti-EJ- and anti-PL7-positive patients compared with patients with anti-PL12, anti-OJ or anti-KS autoantibodies. Raynaud's phenomenon was more frequently observed in patients with anti-PL12 and anti-PL7, and arthritis was less common in the anti-OJ-positive patients. DM rash was found to be more commonly associated with anti-EJ, anti-PL12 and anti-PL7. Additionally, although ILD was frequently found in patients with each of the non-Jo-1 ASAs, patients with anti-EJ or

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anti-PL7 were more likely to develop myositis after presenting with ILD compared to patients with anti-PL12, anti-KS or anti-OJ autoantibodies [23]. These data therefore demonstrate that patients with ASAs share the common features; however, there is a degree of heterogeneity within ASS, with each of the ASAs forming individual subsets.

Studies investigating the use of ASAs as a prognostic marker have demonstrated that anti-Jo-1 autoantibody titres correlate to a moderate degree with serum CK levels and joint and muscle disease activity [24, 25]. Furthermore, the presence of an ASA has been found to predict a good response of ILD to treatment in comparison with ILD in non-ASA myositis patients. Additionally, the results from the RIM study, investigating the treatment response to rituximab in myositis, found anti-Jo-1 autoantibodies to be a strong predictor of clinical improvement compared with the absence of a defined MSA [26, 27]. These data therefore demonstrate that along with helping to classify myositis patients, the ASAs may help to predict disease course and response to treatment.

DM autoantibodies

Anti-Mi-2

Anti-Mi-2 was discovered by Reichlin and Mattioli in 1976 in a 60-year-old lady with DM [28]. Subsequently, anti-Mi-2 was demonstrated to be a specific marker for DM [29] with numerous cohort studies finding anti-Mi-2 in 11–59% of adult DM patients [29–31], as well as 4–10% of JDM patients [32–34]. Clinically, anti-Mi-2 is significantly associated with a range of cutaneous features including Gottron's papules, heliotrope rash, V-sign and shawl sign rashes, and cuticular overgrowth [29, 35].

Generally, patients with anti-Mi-2 autoantibodies have a more favourable prognosis, such as milder muscle involvement and a decreased risk of ILD and malignancy. Furthermore, patients with anti-Mi-2 autoantibodies tend to respond well to immunosuppressive therapy [31, 36]. In addition, the RIM study demonstrated that in anti-Mi-2-positive patients refractory to immunosuppression who were then treated with rituximab, the presence of anti-Mi-2 autoantibodies predicted a shorter time to improvement compared to the absence of these autoantibodies (hazards ratio 2.5, $P < 0.01$) [24].

The autoantigen target has been fully characterized as a nuclear helicase protein, part of the nucleosome-remodelling deacetylase complex involved in gene transcription [37]. Interestingly, the Mi-2 protein has also been reported to have several roles in developmental processes and, in particular, has been demonstrated to be upregulated in regenerating skeletal muscle [38]. It was also demonstrated that Mi-2 expression was significantly higher in muscle samples from DM patients compared with PM patients, suggesting a role for Mi-2 in the disease pathogenesis of DM. This proposal is further supported by data showing that Mi-2 is essential in the repair of the skin basal epidermis [39], linking the autoantigen to cutaneous involvement.

It is interesting that Mi-2 has also been found to be upregulated in human keratinocytes upon exposure to ultraviolet (UV) light [40], making UV light a potential environmental trigger for the initiation of DM. This association has been investigated further; Love *et al.* [41] reported that UV exposure predicted the distribution of anti-Mi-2-positive female patients. However, the association remains inconclusive as serology data from two Mexican cohorts displayed a vast disparity in anti-Mi-2 frequency (43% vs. 14%) despite similar UV exposure [31]. As anti-Mi-2 autoantibodies have a strong association with HLA-DR7 [42], it is feasible that a combination of environmental factors, including UV light, along with a genetic predisposition may help to predict anti-Mi-2 positivity.

Anti-SAE

Anti-SAE autoantibodies target the small ubiquitin-like modifier activating enzyme and were originally described by Betteridge *et al.* [43] in 11 DM patients (8%) from a Caucasian European myositis cohort. Subsequent studies in other European cohorts have reported that the autoantibody occurs at a similar frequency (6–8%) [44, 45] but is less common in Asian cohorts, at approximately 2% [46, 47]. Interestingly, the original study demonstrated a genetic association with the HLA-DQB1*03 haplotype, which may explain the decreased frequency of this autoantibody in non-Caucasian patients. Equally, there appears to be an age-related distribution of this autoantibody, as anti-SAE has only ever been reported in two JDM cases [47].

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All of the anti-SAE studies completed have shown an association with cutaneous involvement and the dermatomyositis phenotype. Furthermore, Betteridge *et al.* and Fujimoto *et al.* have both reported that the skin manifestations commonly develop a few months prior to the onset of muscle weakness [43, 47]. Anti-SAE autoantibodies have no relationship with either malignancy or ILD; however, ILD is generally less common in the European cohorts (0–18%) and much more common in the Asian studies (50–71%), implying a further ethnic difference [43–47].

It has also been suggested that anti-SAE autoantibody positivity may correlate with dysphagia. Betteridge *et al.* found that 78% of anti-SAE-positive patients had dysphagia compared with 43% in the anti-SAE-negative group. Fujimoto *et al.* found that two anti-SAE-positive patients had severe dysphagia and suggested that this condition may be associated with anti-SAE positivity [43, 47].

Anti-TIF1

Autoantibodies to a 155-kDa protein, usually seen with a further 140-kDa band, were identified concurrently by Targoff *et al.* and Kaji *et al.* in 2006/2007 [48, 49]. In subsequent studies, the 155-kDa target was identified as transcription intermediary factor 1 (TIF1) gamma [50]. The 140-kDa protein was later characterized as the TIF1 alpha, with a further 120-kDa target, additionally found in some patients, identified as TIF1 beta [51]. The TIF1 family are tripartite motif-containing proteins and are involved in numerous cellular pathways including cell proliferation, apoptosis and innate immunity [52]. Importantly, TIF1 gamma has also been shown to have a role in tissue differentiation through the inactivation of Smad4 and Smad2/3. This role in tissue differentiation has been further investigated in regenerating skeletal muscle, with high levels of TIF1 gamma expression found in the myonuclei of regenerating myofibres, indicating that TIF1 gamma plays a role in myoblast proliferation and/or regeneration. These findings, in support of those from the previous studies involving Mi-2 and Jo-1 expression, imply that aberrant autoantigen expression may be a driver for myositis pathogenesis [53–55].

Anti-TIF1 gamma has been associated with cutaneous involvement, with DM cohort studies finding anti-TIF1 gamma autoantibodies in 13–31% of adults with DM [48, 49, 56, 57]. This frequency appears to be similar across all ethnic groups and age ranges, with anti-TIF1 gamma also reported in 22–29% of cases in JDM cohorts [48, 56, 58].

Clinically, in adults, anti-TIF1 autoantibodies have been significantly correlated with cancer-associated myositis. A meta-analysis by Trallero-Araguuas *et al.* demonstrated that the sensitivity of anti-TIF1 gamma for diagnosing cancer-associated myositis is 78% [95% confidence interval (CI) 45–94%], with a specificity of 89% (95% CI 82–93%) and positive and negative predictive values of 58% and 95%, respectively [57]. However, whilst anti-TIF1 autoantibodies are as common in children, studies in JDM cohorts have found no association with anti-TIF1 and cancer [56]. It has therefore been proposed that the clinical phenotype of anti-TIF1-positive patients is correlated with age, with older patients having a higher probability of having cancer. This hypothesis is supported by a recent study by Fiorentino *et al.* [52] who demonstrated that age was significantly associated with an increased risk of cancer in anti-TIF1 gamma-positive patients. Additionally, Fujimoto *et al.* [51] suggested that adults with anti-TIF1 gamma autoantibodies should be classified into two subgroups, with the older patients at increased risk of malignancy and muscle weakness and the younger group (25–39 years) having a lower risk of malignancy and being more likely to have a clinically amyopathic DM (CADM) phenotype. This hypothesis is supported by a case report by Matsuura *et al.*, who described anti-TIF1 gamma autoantibodies in four patients, two of whom were young female patients with CADM and no malignancy [59].

Fiorentino *et al.* also investigated additional clinical associations with anti-TIF1 autoantibodies in adult patients. Data showed that patients with anti-TIF1 gamma have a reduced prevalence of Raynaud's phenomenon, arthritis, calcinosis and ILD than anti-TIF1 gamma-negative patients. However, pruritus was found to be positively associated. Anti-TIF1 gamma-positive patients were also found to have lower muscle enzyme levels than the comparator group, which remained significant even after exclusion of the CADM subgroup of patients. In terms of the cutaneous involvement, studies in both juvenile and adult cohorts have demonstrated that anti-TIF1 gamma-positive patients have an increased risk of severe skin

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disease [14, 52, 56], specifically with diffuse photoerythema, with increased numbers of patients developing scalp, facial, V-neck and back rashes. Furthermore, in adults, anti-TIF1 gamma autoantibodies have also been associated with the cutaneous manifestations of psoriasiform lesions, 'red on white' skin changes and hyperkeratotic, verruca-like papules.

Studies on the coexistence of TIF1 gamma, TIF1 beta and TIF1 alpha autoantibodies have been completed in a cohort of 456 DM patients. Fujimoto *et al.* demonstrated that 78 (17%) of these patients had one or more anti-TIF1 antibody. Of those who were TIF1 positive, 29% only had autoantibodies to TIF1 gamma, with a further 62% having both anti-TIF1 gamma and TIF1 alpha autoantibodies. In terms of clinical correlation, it was demonstrated that patients with autoantibodies targeting TIF1 gamma and TIF1 alpha had a malignancy rate of 73%, whereas the anti-TIF1 gamma only group was at significantly less risk (50%, $P < 0.05$). It would therefore be of interest to see whether the proportions of patients reactive to each of these targets vary between JDM, younger and older DM patients [51]. Of note, anti-TIF1 alpha autoantibodies have also been associated with Mi-2. In a study of 108 Japanese DM patients, anti-TIF1 alpha autoantibodies were found in 12 patients; of these, seven had anti-TIF1 gamma autoantibodies and the remaining five patients were also positive for anti-Mi-2 [60]. Although rare, this association between TIF1 and Mi-2 has been reported previously, with two case reports of the existence of anti-TIF1 gamma and anti-Mi-2 autoantibodies [61]. More studies are now required to investigate whether patients with this combination of autoantibodies differ clinically from patients with anti-Mi-2 alone.

Anti-NXP2

Anti-NXP2 autoantibodies, originally termed anti-MJ, were first reported in 18% of patients recruited to a US JDM cohort [62]. Subsequent studies by Targoff *et al.* identified the 140-kDa autoantigen target as nuclear matrix protein 2 (also known as MORC3), a protein involved in transcriptional regulation [63]. Investigations in a UK JDM cohort of 162 patients found anti-NXP2 autoantibodies in 23% of cases, with a significant association with calcinosis [64]. A further cohort study of 64 Argentinian JDM cases found the autoantibody at a similar frequency (25%); however, in this cohort, anti-NXP2 was found to be associated with muscle contractures, atrophy and significant compromise of functional status, with no reported association with calcinosis [58].

More recently, anti-NXP2 has also been described in adult cases. A number of cohort studies have been completed with a wide variation in the frequency of anti-NXP2 autoantibodies. The first report was from a Japanese cohort of 507 patients, where the antibody was described in 1.6% of both PM and DM patients [65]. In a subsequent study by Fiorentino *et al.* [66] anti-NXP2 was found to be much more common, occurring in 17% of DM patients recruited to two adult US myositis cohorts consisting of a total of 213 patients. This frequency (17%) was also seen in an Italian cohort of 58 cases screened by Ceribelli *et al.* [67]. However, further data from the EuMyoNet cohort, consisting of over 1300 Caucasian European adult PM and DM patients, only identified anti-NXP2 autoantibodies in <1% of patients [68]. Because the variations in frequencies are seen across the mainly Caucasian European and US cohorts, it is unlikely that they are due to genetic or environmental differences. The variation in frequency may therefore reflect the methodology used to detect the anti-NXP2 autoantibodies, or the demographic characteristics of the various cohorts.

In terms of clinical associations of anti-NXP2 autoantibodies in adult patients, calcinosis is a much rarer complication in adult-onset myositis compared with JDM [69], and therefore, most of the studies to date have had limited statistical power to test for an association. However, although calcinosis has not been statistically associated, there have been a number of case reports of calcinosis in anti-NXP2-positive adult-onset patients [70]. Additionally, there is evidence to suggest that anti-NXP2 autoantibodies may also be associated with malignancy in adults. The original study by Ichimura *et al.* [65] only contained eight anti-NXP-positive adults; however, three of these patients (38%) had internal malignancies. More recently, Fiorentino *et al.* described anti-NXP2 autoantibodies in 31% of patients with cancer-associated myositis and found a significant association ($P = 0.04$) between anti-NXP2 and cancer in their overall myositis population on univariate analysis. Interestingly, this association had a higher odds ratio (OR) than the well-documented association between anti-TIF1 gamma and malignancy (2.5 vs. 1.9). When the data were stratified by gender and tested by multivariate analysis, anti-

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NXP2 autoantibodies were found to be associated with an increased risk of cancer only in males (OR 5.8, 95% CI 1.4–24.7, $P = 0.02$). Additionally, because it has been demonstrated that the prevalence of cancer in myositis is higher in older patients, the presence of anti-NXP2 and cancer was also analysed by age. The analysis showed no statistical significance; however, there was a trend towards an association, with 55% of anti-NXP2-positive patients above 60 years of age having cancer compared with only 17% of those above 60 years of age without either NXP2 or TIF1 gamma [66].

This potential association with age has been further highlighted by Tansley *et al.* In a study of 285 JDM patients, the authors identified anti-NXP2 autoantibodies in 56 (20%) patients. Clinically, 33% of the overall cohort developed calcinosis during follow-up, with a lower age of onset significantly increasing the risk (OR 0.9, 95% CI 1.10–4.01, $P = 0.025$). The additional presence of anti-NXP2 increased the risk of calcinosis, and this association was still significant when adjusted for age. It is therefore feasible that anti-NXP2 autoantibodies cover a dual spectrum of clinical associations, with calcinosis being more significant in younger patients and cancer being the more common complication in older adults [71].

Anti-MDA5

Autoantibodies to MDA5 (originally termed p140) were first identified in a Japanese cohort in 2005 [72]. Two groups subsequently identified the autoantigen target as melanoma differentiation-associated gene, a cytoplasmic protein with a role in the recognition of viral RNA, which forms part of the innate immune system [73, 74]. Anti-MDA5 autoantibodies were originally found in eight of 42 patients (19%) in an Asian adult DM cohort and were found to be associated with CADM and rapidly progressive interstitial lung disease (RP-ILD). Nakashirma *et al.* further demonstrated that due to this association with RP-ILD, anti-MDA5 autoantibodies were a marker of a poor prognostic outcome, reporting that 46% of anti-MDA5-positive patients died of respiratory failure within 6 months of disease onset [74]. Interestingly, whereas a meta-analysis of numerous Japanese, Korean and Chinese studies demonstrated the utility of anti-MDA5 for identifying RP-ILD in myositis patients, the same association has not been demonstrated for US and European cohorts. Fiorentino *et al.* [75] investigating anti-MDA5 autoantibodies in a US cohort, demonstrated an association between anti-MDA5 and ILD; however, RP-ILD was not found to be significant. Similar findings were also reported by Betteridge *et al.* in a 1500-case European myositis study and by Hall *et al.* in an additional US cohort [76, 77].

Furthermore, whilst studies have demonstrated that anti-MDA5 autoantibodies occur in 10–48% of DM patients in East Asian cohorts [78], the frequency is substantially lower in US and European cohorts, only being described in 0–13% of DM patients [44, 75, 77, 79]. Overall, these discrepancies imply that either a genetic or an environmental factor is associated with anti-MDA5 autoantibody generation. This implication is supported by an epidemiological study by Muro *et al.* [80] who showed that, in central Japan, there is an increased prevalence of anti-MDA5-positive patients in two areas along the Kiso River, suggesting an environmental influence. Equally, the findings by Gono *et al.* [81] of an HLA-DRB1*0101/*0405 association supports an argument for a genetic predisposition of anti-MDA5 positivity.

The detailed cutaneous DM associations of anti-MDA5 autoantibodies have been further analysed by Fiorentino *et al.* [75] in a study of 77 DM patients. The authors found anti-MDA5 autoantibodies in 13% of patients, and in comparison with the MDA5 autoantibody-negative group, anti-MDA5 was found to be associated with the cutaneous manifestations of hand swelling, arthritis, skin ulceration, palmar papules, mechanics hands, panniculitis, alopecia and oral ulcers. In agreement with previous reports, they also found that anti-MDA5 was associated with CADM.

Anti-MDA5 autoantibodies have also been reported in JDM cohorts. In the initial study by Kobayashi *et al.* [82] anti-MDA5 autoantibodies were identified in five of 13 overall JDM patients and in five of six JDM patients with ILD, inferring similar clinical associations of anti-MDA5 in juvenile and adult patients. This is supported by a further comprehensive study by Tansley *et al.* in 285 UK JDM patients. The authors found anti-MDA5 autoantibodies in 21 patients (7%) and, similar to the US adults, demonstrated significant associations with skin and mouth ulceration, arthritis and milder muscle disease. Furthermore, 19% of the anti-MDA5-positive JDM patients had ILD although, similar to the

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European and US adult anti-MDA5-positive patients, this was not rapidly progressing [83].

In East Asian adults and children, anti-MDA5 titres have been demonstrated to correlate with the risk of developing RP-ILD and subsequent survival rates [82, 84, 85]. The autoantibodies have also been shown to disappear during disease remission, implying that they are a good marker of disease course [86]. However, contrary to these results, Hall *et al.* reported no correlation between serial autoantibody titres and clinical course in a cohort of Caucasian anti-MDA5-positive patients [77]. Further studies are therefore required to investigate the clinical utility of serial sample testing in patients with anti-MDA5 autoantibodies.

Immune-mediated necrotizing myopathy autoantibodies

Anti-SRP

Autoantibodies to signal recognition particle (SRP) were first described by Reeves *et al.* in 1986 [87]. The cytoplasmic SRP autoantigen is an RNP complex involved in the recognition and transportation of newly synthesized proteins to the endoplasmic reticulum [88]. Studies have demonstrated that anti-SRP autoantibodies occur in approximately 5% of Caucasian adult patients with IIM [89, 90], with higher frequencies in Asian cohorts (8–13%) and African American patients [41, 42, 91]. These variations in frequency therefore imply a genetic association, and Love *et al.* reported that anti-SRP patients are more likely to have a DR5 HLA genotype compared with ASA-positive patients [42].

Clinically, anti-SRP-positive patients have a decreased likelihood of cutaneous involvement, with the autoantibody being associated with the PM phenotype [42, 89]. Anti-SRP autoantibodies have been shown to be significantly associated with severe necrotizing myopathy, with patients rapidly developing progressive muscle weakness and severe debilitation within months of onset [89]. Systemically, anti-SRP autoantibodies have also been reported to be associated with an increased risk of dysphagia [92]. Anti-SRP positivity was originally correlated with cardiac involvement [42]; however, this finding has not been replicated in other larger cohorts [90, 92], leaving this association still unresolved.

Anti-SRP positivity was initially associated with an increased mortality rate [42], although in a more recent study no significant difference was found in the survival rates of anti-SRP-positive patients compared with either anti-ASA-positive or anti-ASA-negative PM controls [92]. However, despite the similar survival rates, anti-SRP-positive patients are generally reported to be refractory to conventional immunosuppressive therapy compared to other autoantibody subgroups [92–94]. Furthermore, a case report by Whelan and Isenberg also suggested that anti-SRP patients may respond poorly to B-cell depletion [95], making anti-SRP a marker of a poor prognostic outcome.

Anti-SRP autoantibodies have also been described in juvenile patients, although due to the fact that juvenile PM itself is extremely rare, there have only been limited numbers of case reports. Interestingly, however, the increased prevalence in African Americans is also seen in juvenile PM, along with the associated clinical manifestations [88].

Patients with anti-SRP autoantibodies have significantly raised CK levels at diagnosis [90]; however, although this level is not predictive of the degree of muscle weakness, studies have demonstrated a correlation between CK levels and anti-SRP titres over time. Furthermore, changes in anti-SRP autoantibody levels have also been shown to correlate with an improvement in muscle strength [96, 97]. It is therefore feasible that the testing of anti-SRP autoantibodies titres throughout the disease course may be helpful for monitoring disease progression.

Anti-HMGCR

Autoantibodies to a 200-kDa/100-kDa complex were first described in 64% of necrotizing myopathy patients without any known MSA/MAA [98]. Further studies identified the autoantigen target as 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMGCR), the rate-controlling enzyme of the cholesterol-producing mevalonate pathway. Studies have demonstrated the DRB1*11:01 haplotype to infer an increased risk of anti-HMGCR positivity in both Caucasian (OR 24.5, $P = 3.2 \times 10^{-10}$) and African American (OR 56.5, $P = 3.1 \times 10^{-6}$) patients, with DQA1 and DQB1 being conversely protective [99]. Patients with anti-HMGCR autoantibodies have been characterized by an increased risk of muscle weakness, elevated CK levels and myopathic changes on EMG. Furthermore, following the development of quantitative ELISAs, it has been shown that anti-HMGCR titres at disease onset correlate with CK levels and leg or arm

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strength, with subsequent clinical improvements correlating with a decrease in anti-HMGCR autoantibody levels [68]. Because patients with anti-HMGCR autoantibodies have been shown to respond well to immunosuppression, but have a tendency to relapse upon weaning of therapy, anti-HMGCR titres may therefore not only act as a diagnostic tool, but also serve as a prognostic marker of disease course [100].

Anti-HMGCR autoantibodies were originally reported to be significantly associated with statin exposure, with 63–67% of US anti-HMGCR-positive patients having a prior history of statin use [98, 101]. This association was shown to be more pronounced in older patients, with Mammen *et al.* reporting 92% of over 50-year-old anti-HMGCR-positive patients having a statin exposure compared with only 25–37% of DM, PM and inclusion body myositis (IBM) patients [101]. Furthermore, studies in nonmyopathic statin users demonstrated anti-HMGCR autoantibodies to be myositis specific, with no patients from a control cohort of 1966 statin users developing anti-HMGCR autoantibodies [102]. However, whilst this autoantibody has been associated with statin use, studies have also demonstrated the presence of anti-HMGCR autoantibodies in statin-naïve patients. Reports from European and Japanese cohorts showed that only a minority of anti-HMGCR-positive patients (38–44%) had a prior statin exposure. As anti-HMGCR autoantibodies have also been detected in statin-naïve juvenile patients, it has been proposed recently that anti-HMGCR autoantibodies are a marker of necrotizing myopathy as opposed to statin-induced myositis [103, 104].

IBM autoantibodies

Anti-cN1A

Inclusion body myositis has been traditionally viewed as a degenerative myopathy with secondary inflammation, rather than a primary autoimmune disease. Supporting this hypothesis, until recently, only a small proportion (17–43%) of IBM patients were routinely found to be autoantibody positive, with the majority of these cases involving MAAs as opposed to MSAs [105]. However, in 2011, two groups simultaneously described novel autoantibodies in IBM, with Salajegheh *et al.* reporting a 43-kDa target and Pluk *et al.* reporting autoantibodies targeting a 44-kDa protein [106, 107]. These novel autoantibodies were found to target the same autoantigen, cytosolic 5'nucleotidase 1A (cN1A), which is a protein involved in the hydrolysis of adenosine monophosphate, leading to physiological control of energy balance, metabolic regulation and cell replication.

Further studies by both groups demonstrated highly elevated reactivity of anti-cN1A autoantibodies in 33–34% of IBM patients, with low cross-reactivity in the control groups (4–5% PM, 0–4% DM, 0–3% neuromuscular disorders and 0% in healthy controls) [108, 109]. More recently, a study investigating the prevalence of anti-cN1A autoantibodies in other autoimmune diseases demonstrated these autoantibodies also in 36% of patients with Sjögren's syndrome and 20% of patients with SLE, decreasing the overall specificity of this autoantibody for myositis. However, although analysis of anti-cN1A-positive versus anti-cN1A-negative IBM patients showed no correlation with age, duration of symptoms, weakness or antinuclear autoantibody or MAA status, the rarity of anti-cN1A in PM and DM patients still makes this autoantibody a key marker for differentiating between myositis subtypes [110].

Conclusions

In this review, we have highlighted recent studies identifying myositis autoantibodies and their clinical associations. With greater numbers of myositis patients being demonstrated to be MSA or MAA positive, it is clear that myositis autoantibodies have an increasing utility as both diagnostic and prognostic biomarkers. The presence of an MSA/MAA autoantibody can aid in the classification of myositis patients into more homologous groups than the traditional PM and DM subtypes, predicting further disease complications and possible responses to treatment. Further studies are now required to identify novel autoantigenic targets in patients who are currently regarded as autoantibody negative and to understand the role of these autoantibodies and their autoantigens in the pathogenicity of myositis.

Conflict of interest statement

The authors declare no conflict of interests.

Acknowledgements

ZB is funded by the Bath Institute for Rheumatic Diseases and Myositis, UK, and by Association Française contre les Myopathies. We also acknowl-

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edge the European Science Foundation, for supporting EuMyoNet.

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