Invited critical review

Discovery of new biomarkers of idiopathic inflammatory myopathy

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Abstract

Idiopathic inflammatory myopathies (IIMs) are a group of acquired diseases, characterized by immune-inflammatory processes primarily involving skeletal muscle. According to recent classification criteria, five major diseases have been identified: polymyositis (PM), dermatomyositis (DM), immune-mediated necrotizing myopathy (IMNM), juvenile idiopathic myositis (JIM) and sporadic inclusion body myositis (sIBM). Although the etiology of IIMs is still incompletely understood, there is much evidence supporting the involvement of genetic, immunological, and environmental factors. In recent years, many new biomarkers have been identified as useful indicators for diagnosis, disease subtypes, prognosis, or response to treatment of IIMs. This article reviews the new biomarkers in serum and muscle tissue, focusing on their pathogenic, diagnostic and prognostic value in IIM. We assigned value based on the categories of myositis specific autoantibodies, cytokines, and genetic markers.

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Idiopathic inflammatory myopathies (IIMs) are a group of clinically heterogeneous, inflammatory muscle disorders characterized by proximal and symmetric muscle weakness and multisystem involvement. The main clinical phenotypes of IIMs are classified as polymyositis (PM), dermatomyositis (DM), immune-mediated necrotizing myopathy (IMNM), sporadic inclusion body myositis (sIBM), and juvenile idiopathic myositis (JIM) [1]. The clinical manifestations vary widely in individual IIM patients. The muscle weakness develops over weeks to months in PM and DM, or insidiously over months to years in sIBM. However, the onset could be acute over days or weeks in IMNM. IBM also affects the distal muscles and could be asymmetric. Muscle inflammation is the histologic hallmark for all the IIM subtypes, but the site of inflammation, and the type of cells involved, are different for each subtype. In PM and IBM, the main inflammatory cells consist of T cells, which are located in endomysia, and surround the normal muscle fibers expressing the MHC-I molecules, resulting in muscle fiber necrosis. In DM, the inflammation is predominantly perivascular or in the intermuscular septa and around, which contributes to perifascicular atrophy. In IBM, besides the inflammation, there are rimmed vacuoles and congophilic amyloid deposits within the vacuoles. IMNM is a necrotizing myopathy, which is absent or rare in inflammatory cells while the MHC-I molecule expression is spotty and mostly on necrotic fibers. Besides muscle manifestation, another significant characteristic of DM are skin rashes, which include heliotropic rashes, often associated with periorbital edema and papular erythematous rashes over the knuckles (Gottron papules). In addition, an erythematous, macular, sun-sensitive rash may appear on the face, neck and anterior chest (V-sign), shoulders and upper back (shawl sign), hips (holster sign), and extensor surfaces of the elbows, knuckles, knees, and malleoli (Gottron sign).

Over the past decade, enthusiasm and efforts have been devoted to tackling numerous challenges ranging from understanding the etiopathogenesis of IIMs, through the development of diagnostic tests and biomarkers, to improving care for IIM patients. Autoantibodies are etiopathogenesis of IIMs, through the development of diagnostic tests and biomarkers, to improving care for IIM patients. Autoantibodies are evidence of muscle weakness, significant elevated serum creatine kinase (CK) levels, but no earlier onset of PM/DM or calcinosis.

IIMs is another area of concern [4–6]. Recently, several experiments suggested that susceptibility to IIMs likely has a polygenic cause, which involves interactions between physiological and environmental factors.

In this article, we present a brief review of newly discovered biomarkers of IIMs, focusing on MSAs, cytokines, and genetic factors. We also summarize their roles in diagnosis, monitoring, stratification, and prediction of response to therapy.

2. Myositis-specific autoantibodies (MSAs)

2.1. Anti-nuclear matrix protein (NXP)-2 autoantibody

The presence of an autoantibody targeting a 140 kDa protein in the sera of patients with juvenile DM (JDM), called anti-MJ/P140 antibody, was first described by Oddis in 1997. This autoantibody was found in 18% of JDM patients but was not seen in juvenile overlap myositis or other connective tissue diseases [7]. A preliminary study by Targoff and colleagues identified the autoantigen of anti-MJ/P140 antibody to be nuclear matrix protein-2 (NXP-2) following an immunoprecipitation assay [8], also known as MORC3, which is localized in promyelocytic leukemia nuclear bodies and plays an important role in RNA metabolism and in maintaining nuclear structure through activating the p53 pathway to regulate the transcription of target genes [9]. The frequency of the anti-NXP-2 antibody is approximately 23–25%, according to two subsequent studies on JDM cohorts in the UK and Argentina, and this specific autoantibody was significantly associated with the incidence of calcinosis—defined as the abnormal deposition of calcium salts in a part or tissue of the body [10,11]. In addition, the study involving the UK cohort established that the presence of the HLA-DRB1*08 allele may contribute to genetic susceptibility for the development of anti-NXP-2 antibody in a Caucasian pediatric population [10].

More recently, Ceribelli and colleagues reported that the anti-NXP-2 antibody can also be detected in adult patients with a frequency of 30% and 8% in PM and DM patients, respectively [12]. Patients who tested positive for the anti-NXP-2 antibody more commonly experienced earlier onset of their disease, heliotropic rash, and calcinosis but a good response to therapy, uncomplicated with heart or lung involvement or malignancy. However, Ichimura [13] found that adult Japanese patients with PM and DM had a lower frequency of anti-NXP-2 antibodies. They found that only 1.6% of 507 PM and DM patients had the anti-NXP-2 antibody. All the patients with the anti-NXP-2 antibody showed evidence of muscle weakness, significantly elevated serum creatine kinase (CK) levels, but no earlier onset of PM/DM or calcinosis.

Table 1

<table>
<thead>
<tr>
<th>Autoantibody</th>
<th>Target antigen</th>
<th>Clinical association</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-NXP-2</td>
<td>Nuclear matrix protein-2</td>
<td>JDM; DM with calcinosis; CAM</td>
<td>23–25% in JDM; 1.6–30% in PM/DM [8,10–14]</td>
</tr>
<tr>
<td>Anti-MDA5</td>
<td>Melanoma differentiation-associated gene 5 protein</td>
<td>CADM; CADM with RP-ILD in Asian; DM with mild ILD in Caucasian; JDM with ILD; skin lesions</td>
<td>6.9–26% in DM; 37.5–65% in CADM patients [15,17,18,20–28]</td>
</tr>
<tr>
<td>Anti-TIF1</td>
<td>Transcriptional intermediary factor 1-α, β, γ</td>
<td>CAM; typical DM skin rashes</td>
<td>13–21% in DM; 50–64.3% in adult CAM; 23% in JDM [29,30,34–39]</td>
</tr>
<tr>
<td>Anti-SAE</td>
<td>Small ubiquitin-like modifier activating enzyme</td>
<td>DM with severe skin rash</td>
<td>7–8% in Caucasian DM; 1.5–1.8% in Japanese DM [40–44]</td>
</tr>
<tr>
<td>Anti-HMCR</td>
<td>3-Hydroxy-3-methyl coenzyme A reductase protein</td>
<td>Statins-associated autoimmune myopathy; IMNM</td>
<td>40% in statins-associated autoimmune myopathy; 24% in IMNM [45–50]</td>
</tr>
<tr>
<td>Anti-CTN1A</td>
<td>Cytoplasmic 5′-nucleotidase</td>
<td>sIBM</td>
<td>33–52% in sIBM; 4.2% in PM; 4.5% in DM [51–53]</td>
</tr>
</tbody>
</table>

JDM, juvenile dermatomyositis; PM, polymyositis; DM, dermatomyositis; CADM, clinically amyopathic dermatomyositis; CAM, cancer-associated dermatomyositis; IMNM, immune-mediated necrotizing myopathy; sIBM, sporadic inclusion body myositis; RP-ILD, rapidly progressive interstitial lung disease; ILD, interstitial lung disease.
Additionally, cancer-associated myositis (CAM) was observed in 37.5% of patients in the Japanese cohort carrying the anti-NXP-2 antibody. A recent study by Fiorentino involving a US cohort demonstrated that most patients with CAM had anti-NXP-2 antibodies [14]. However, these results from different studies are contradictory. Therefore, extensive and large-sample studies are required to validate the prevalence and clinical association proposed for the anti-NXP-2 antibody in IIM.

### 2.2. Anti-melanoma differentiation–associated gene 5 (MDA5) autoantibody

In 2005, Sato and colleagues [15] first identified a novel autoantibody that recognized a 140 kDa protein by immunoprecipitation and immunoblotting in eight Japanese adult patients with clinical amyopathic DM (CADM). Of these patients, 50% had rapidly progressive interstitial lung disease (ILD). Subsequently, they confirmed that the autoantigen targeted by anti-CADM-140 antibodies were intracellular proteins encoded by melanoma differentiation associated gene 5 (MDA5). The MDA5 protein is a cytoplasmic RNA helicase that binds to the retinoic acid–inducible gene 1 (RIG-1) family members. During a viral infection, the MDA5 protein detects and binds to viral RNA to trigger defenses against the virus. Moreover, viral infections have been considered as potential etiologies for IIM in previous studies. The antiviral response could have induced an autoimmune response to MDA5 and produced an anti-MDA5 autoantibody, which may further contribute to the pathogenesis of CADM and rapidly progressive ILD [16].

In the next few years, several Japanese researchers reported the prevalence and the clinical significance of the anti-MDA5 autoantibody in Japanese patients. Hoshino [17] detected anti-MDA5 autoantibody in 80 patients with DM and found that the autoantibodies were positive in 26% of DM and 65% of CADM patients. Additionally, 95% of anti-MDA5-positive patients had ILD, while 79% of them had rapidly progressive ILD (RP-ILD). Koga [18] stated that the anti-MDA5 antibody was highly prevalent among patients with CADM complicated with skin ulcers and RP-ILD. Patients carrying anti-MDA5 also had significantly lower 6-month and 5-year survival rates. Gono [19] showed that anti-MDA5 antibody titers decreased when the CADM-ILD went into remission after treatment. In contrast, anti-MDA5 antibody levels increased in the non-response group. They suggested that assessing the anti-MDA5 antibody could be useful for predicting therapeutic effect. In 2012, Cao et al. investigated the association of clinical features with anti-MDA5 antibody in Chinese patients with DM. The prevalence of anti-MDA5 was 37.5% in CADM. Skin ulcers and ILD were significantly more common in patients carrying anti-MDA5 than in antibody-negative patients. In the same year, Chen and colleagues [20] analyzed the anti-MDA5 antibody in 113 adult Chinese IIM patients. They found that the frequency of the anti-MDA5 antibody in DM and CADM patients were 22.6% and 62.5%, respectively. The anti-MDA5 antibody had a strong association with acute/subacute ILD. The presence of the anti-MDA5 antibody led to serious clinical consequences and was an independent risk factor for the development of ILD in DM patients. A study involving a Korean cohort also observed a strong association between the anti-MDA5 antibody and DM with RP-ILD [21].

Consistent with studies on Asian cohorts, recent studies on US patients revealed a distinct anti-MDA5 phenotype. Hall et al. [22] reported that the anti-MDA-5 antibody was detected in 11 of 160 patients (6.9%) with DM. Although 72.7% of those patients demonstrated ILD, RP-ILD was not observed among anti-MDA5–positive patients, and generally, these patients experienced mild ILD and responded well to immunosuppressive treatments compared to patients in the Asian cohorts. In addition, these patients more commonly suffered myopathy and symmetric polyarthritis. Fiorentino [23] found that 13% of patients with DM had circulating anti-MDA5 antibodies, and all patients had a high prevalence of skin lesions but a low frequency of ILD; only 22.2% of antibody-positive patients had RP-ILD. Patients with anti-MDA5 antibody had no increased risk for ILD. Narang et al. [24] identified a strong association between anti-MDA5 antibodies and cutaneous ulcers. Furthermore, the anti-MDA5 antibody was associated with ILD only in patients with cutaneous ulcers. However, Labrador-Horrillo et al. [25] detected the anti-MDA5 antibody in adult Mediterranean patients with DM and discovered the association between this autoantibody and RP-ILD in their cohort.

Kobayashi et al. first confirmed that the anti-MDA5 antibody had predictive value for diagnosis of JDM with ILD in 2011 [26]. They investigated 13 JDM patients and detected the anti-MDA5 antibody in all of them; five of those patients had ILD. Shah et al. [27] demonstrated that exposure to ultraviolet light positively correlated with anti-MDA5–associated JDM. In one JDM cohort in the UK, Tansley [28] reported that anti-MDA5 antibodies could be identified in a small but significant proportion of patients with JDM, classifying this group as a distinctive clinical subset.

Taken together, the results of these studies demonstrated that anti-MDA5 autoantibody would be a useful marker for the diagnosis of DM, especially for the CADM/RP-ILD subset among Asian cohorts. However, further study is needed to understand how this specific autoantibody distinguishes clinical phenotypes and predicts outcomes in both adult and juvenile patients.

### 2.3. Anti-transcriptional intermediary factor (TIF) 1-γ autoantibody

In 2006, Targoff et al. first discovered an autoantibody that recognized 155 kDa and 140 kDa proteins by performing radiolabeled IP assays of the sera of juvenile and adult patients with DM. The anti-P155/140 antibody occurred in 21% of adult DM patients and 29% of JDM patients. Six of eight adult patients (75%) with cancer-associated DM (CAM) had anti-P155/140 antibody [29]. Subsequently, they identified the antigen target proposed to be a transcriptional intermediary factor 1 (TIF-1)–γ protein, which belongs to a tripartite motif-containing family of proteins consisting of at least three members: TIF-1-α, TIF-1-β, and TIF-1-γ.

In 2007, Kaji et al. [30] reported that the anti-TIF1-γ antibody was found in seven of 52 (13%) Japanese patients with DM. The frequency of skin lesions—such as heliotrope rash, Gottron’s papules, and erythema—was significantly higher in antibody-positive patients than in patients without the antibody. Among antibody-positive patients, 71% had CAM, but none of them presented with ILD. Moreover, Chinoy et al. [31] demonstrated that 50% of CAM patients had anti-P155/140 antibody, and 97% of patients without anti-P155/140 antibody did not have CAM in a UK cohort. The anti-P155/140 antibody had a high negative predictive value in the diagnosis of CAM. Later, in several Asian cohorts and in one Spanish cohort, the relationship between anti-P155/140 antibody and CAM was confirmed in DM patients [17,32]. Recently, a meta-analysis was performed to assess the association of anti-TIF-1-γ antibody with CAM. Findings from this review article including 6 relevant studies, a total of 312 adult IIM patients, suggested that the sensitivity and positive predictive values of anti-P155/140 antibody for the diagnosis of CAM were 78% and 58%, respectively. The specificity and negative predictive values were 89% and 95%, respectively [33].

In 2012, Fujimoto [34] determined that the 155 kDa protein recognized by the anti-P155/140 autoantibody was TIF1-γ, and that the anti-P140 was identical to TIF1 α. In addition, the anti-P155/140 antibody also targeted TIF1-β. The anti-TIF1 family proteins occurred in a small but significant proportion of patients with JDM, classifying this group as a distinctive clinical subset. Satoh et al. [35] estimated that DM patients with anti-TIF1-α antibody also carry anti-Mi-2 antibody, who were characterized by classical DM without cancer. Satoh et al. [36] investigated DM patients with anti-TIF1-β antibody had mild myositis but none of them developed malignancy. More recently, Lu et al. [37] investigated 211 adult Chinese patients with PM and DM. The anti-P155/140 antibody can recognize all TIF1 family proteins. The frequencies of the anti-TIF1-α, anti-TIF1-β, and anti-TIF1-γ antibodies in CAM patients were 42.9%, 0%, and 64.3%, respectively.
The sensitivity of detection of both the anti-TIF1-α and anti-TIF1-γ antibodies in the diagnosis of CAM in adult DM patients was significantly higher than that of anti-TIF1-γ alone or of anti-TIF1-α alone. However, the clinical features observed in juvenile myositis patients with anti-P155/140 were distinct from those of adult patients. In a study based on a cohort of British and Irish patients, Gunawardena et al. [38] found that the anti-P155/140 antibody was present in 23% of DM patients, and that the antibody appeared more often in male patients. Typical skin rashes—such as Gottron's papules and skin edema—were significantly more common in the antibody-positive group than in the antibody-negative group, but no correlation was observed between the anti-P155/140 antibody and tumors in juvenile myositis patients. Another study evaluating the myositis autoantibody profiles of a US JIM cohort showed that anti-P155/140 was the most common MSA identified in juvenile myositis patients [39]. Gottron's sign was a significant clinical feature, which was similar to Gunawardena's study. However, no positive correlation between the presence of anti-p155/140 autoantibody and the clinical manifestations of edema or ulceration was shown in their data.

All these studies indicate a strong association between the anti-TIF1 antibody and CAM. They also suggest that DM patients who have anti-TIF1-γ antibody should undergo screening for cancer. Further study is needed to clarify whether and how TIF1 family proteins and the anti-TIF1 autoantibodies contribute to the pathogenesis of CAM.

2.4. Anti-small ubiquitin-like modifier activating enzyme (SAE) autoantibody

Anti–small ubiquitin-like modifier (SUMO) activating enzyme (SAE) was first reported as a candidate MSA in 2007. Betteridge et al. [40] detected sera from 20 adult DM patients using Hep-2 indirect immunofluorescence (IF) and found that sera from two patients revealed a speckled nuclear-sparing pattern. Through subsequent IP and mass spectrometry assays, they found that both patients’ sera banded at 40 kDa and 90 kDa proteins corresponding to SAE1 (38 kDa) and SAE2 (71 kDa), which is a heterodimer of two subunits of SAE and was previously identified as having a key role in post-translational modification and is associated with many inflammatory diseases. These findings were absent in other diseases and healthy controls. In this study, they also observed that both DM patients had similar clinical features of severe skin involvement, dysphagia, and limited ILD. In 2009, the same group analyzed the anti-SAE autoantibody in a large UK cohort containing 266 IIM patients, 250 other connective tissue diseases patients, and 50 healthy controls [41]. The overall prevalence of the anti-SAE autoantibody among the IIM patients was 4%, while the frequency among the DM patients was 8%. Skin lesions were more common in DM patients who carried the anti-SAE autoantibody. Of these patients, 82% experienced heliotrope rash and Gottron’s sign, and 78% presented with dysphagia and muscle weakness, whereas very few of them presented with malignant cancer or ILD. Furthermore, the presence of anti-SAE autoantibody appears to be associated with HLA class II haplotypes.

In another study, Tarricone [42] investigated the association between the anti-SAE autoantibody and clinical features of an Italian cohort. The prevalence of the anti-SAE autoantibody in Italian patients with DM was 7%. Similarly, patients with positive anti-SAE autoantibody are more likely to develop skin lesions, as it was observed in Betteridge’s study. In contrast, dysphagia, muscle weakness, and ILD appeared to be absent in the Italian cohort. In recent studies done by Muro and Fujimoto [43,44], there was low prevalence of the anti-SAE autoantibody in adult Japanese DM patients. Muro found a prevalence of 1.8%, and Fujimoto found a 1.5% prevalence rate among DM patients who carried the anti-SAE autoantibody. All patients with positive antibody had skin rashes complicated with or without dysphagia. Among these cases, CAM was observed in one patient in the Muro study, and a high frequency of ILD (71%) was reported in the Fujimoto study. Although the prevalence of the anti-SAE autoantibody is different between Asian and Caucasian patients, similar clinical phenotypes of cutaneous manifestations suggested that the anti-SAE autoantibody may be a novel serological biomarker for DM in addition to the anti-Mi-2 autoantibody, which has been previously confirmed as a highly specific MSA for DM.

2.5. Anti-3-hydroxy-3-methyl coenzyme A reductase protein (HMGCR) autoantibody

In 2010, Christopher-Stine et al. [45] identified a novel autoantibody against unknown 100 kDa and 200 kDa proteins in the sera of 16 of 225 myopathy patients. All of the patients with the anti-P200/100 autoantibody shared similar clinical features, including proximal weakness, extremely elevated CK levels, and abnormal muscle electrical tests. Of these 16 patients, 63% had been exposed to statins prior to developing clinical symptoms. In addition, most muscle biopsies obtained from these patients showed predominantly necrotizing myopathies with an overexpression of MHC class I on the sarcolemma of non-necrotic fibers, muscle fiber necrosis, and a few inflammatory infiltrations. Therefore, they considered anti-P200/100 autoantibody may represent a unique subset of IIM: immune-mediated necrotizing myopathy (IMNM).

One year later, the same group confirmed via IP assay that sera from anti-P200/100–positive patients could recognize the intracellular C-terminal domain of 3-hydroxy-3-methyl coenzyme A reductase (HMGCR) with a molecular weight of 100 kDa, while the 200 kDa protein immunoprecipitated with HMGCR, perhaps is an HMGCR homodimer [46]. Subsequently, they developed an ELISA assay for detecting the anti-HMGCR autoantibody in 750 myopathy patients and found that the frequency of anti-HMGCR autoantibodies in myopathy patients was 6%. All anti-HMGCR–positive patients experienced the typical clinical features of IMNM, and 66.7% of them had a history of statin intake. They also found that expression of HMGCR was up-regulated in regenerating muscle fibers in vivo, and that statins could stimulate the expression of HMGCR in muscle fibers in vitro. Therefore, they suggested that statin-induced anti-HMGCR production may contribute to the pathogenesis of IMNM [46]. However, a further population-based study in 2012, which investigated the prevalence of anti-HMGCR in participants—including 763 currently using statins, 322 taking other cholesterol-lowering medications, and 881 never having used cholesterol-lowering agents—they found no significant differences of serum anti-HMGCR titers among statin current users, past users, and non-users. There were no statistically significant differences between the anti-HMGCR levels of statin-intolerant patients and those with self-limited statin-associated musculoskeletal side effects [47]. Taken together, these findings indicate that the anti-HMGCR autoantibody is specific to patients with an autoimmune myopathy.

In the same year, Werner et al. [48] reported the correlation between anti-HMGCR autoantibody levels and disease activity. They found that 55 of 1006 patients who were suspected myopathies (5.5%) were positive for the anti-HMGCR autoantibody, 40 of whom were statin-exposed. Serum levels of the anti-HMGCR antibody were associated with elevated CK levels and muscle strength. In addition, statin-exposed individuals responded better to therapy; their decreased anti-HMGCR titers were associated with decreased CK levels and improved muscle strength compared to the therapy responses of subjects who did not take statins. Werner et al. suggested that serum anti-HMGCR levels may be an indicator of disease activity among patients with statin-associated anti-HMGCR myopathy.

More recently, Drouot and colleagues [49] studied 150 patients with IMNM and found that 24% of patients with IMNM were positive for the anti-HMGCR antibody; only 40% of antibody-positive patients had been exposed to statins. Anti-HMGCR titers correlated positively with CK levels and muscle strength. Yves Allenbach [50] later studied a larger cohort and confirmed the relationship between the anti-HMGCR autoantibody and IMNM. The majority of patients were statin-naïve and, therefore, required immunosuppressive treatments. In contrast to previous studies, the heterogeneous results of anti-HMGCR antibodies...
are not always associated with the use of statins, suggesting that further studies are required to clarify the correlation between the clinical and serological phenotype in patients with IMNM.

### 2.6. Anti-cytoplasmic 5′-nucleotidase 1 (cN1A) autoantibody

In previous studies, sIBM had been identified as having different serological features and a lack of circulating autoantibodies in contrast with other subsets of IBM. In 2011, Salajegheh et al. [51] analyzed the interactions between the sera of sIBM patients and lysates of human skeletal muscle cells by immunoblotting, and found that serum samples from 52% of sIBM patients could recognize an approximate 43 kDa muscle protein, while no positive reaction was detected in sera from PM, DM or myasthenia gravis patients or healthy controls. They concluded that the autoantibody may be a novel biomarker for sIBM. Two years later, Pluk and colleagues [52] identified a 44 kDa skeletal muscle protein (Mup44) as a specific autoantibody target in sIBM patients. They subsequently confirmed that the target antigen of anti-Mup44 autoantibody was cytoplasmic 5′-nucleotidase 1 (cN1A) by mass spectrometric analyses and immunoprecipitation assays. Simultaneously, Larman and Salajegheh et al. [53] also determined that cytosolic 5′-nucleotidase 1A (cN1A or NT5C1A) was an sIBM autoantigen against the anti-43 kDa protein by using mass spectrometry and phage immunoprecipitation sequencing. This protein is abundant in human skeletal muscle, catalyzing nucleotide hydrolysis into nucleosides, and is involved in a variety of cellular functions, such as metabolic regulation, degradation of nucleotides, and DNA repair. Immunohistochemical staining showed that cN1A is localized in rimmed vacuoles and in the perinuclear region in degenerating muscle fibers. These histopathologic features suggest that the cN1A protein and the anti-cN1A autoantibody may be involved in the degradation of sIBM muscle and the pathogenic progress of sIBM.

In these two studies, Pluk and colleagues [52] demonstrated that the occurrences of the anti-cN1A autoantibody in sIBM, PM, DM, and other neuromuscular disorders were 33%, 4.2%, 4.5%, and 3.2%, respectively. Meanwhile, they observed high concentrations of anti-cN1A autoantibodies in sIBM sera but low concentrations in the sera of patients with DM, PM, and other neuromuscular disorders. Larman [53] showed that the sensitivity and specificity of the moderate reactivity of anti-cN1A autoantibody were 70% and 92%, respectively, while the high reactivity of anti-cN1A was 34% sensitive and 98% specific for the diagnosis of sIBM. There were no correlations between anti-cN1A autoantibody level and clinical features such as age, long disease course, muscle strength, and the presence of ANA. These recent studies suggest that the anti-cN1A autoantibody may be a helpful serological biomarker for the diagnosis of sIBM. However, these tests were only performed in a few studies. Further investigation is needed to confirm whether this autoantibody is or can be a valid and reliable clinical diagnostic tool of sIBM.

### 3. Cytokines

#### 3.1. IL-6

Interleukin-6 (IL-6) is a pleiotropic cytokine that regulates multiple responses, such as immune responses, acute phase reactions and hematopoiesis [54]. IL-6 gene expression could be weakly detected in muscle specimens from patients with myositis by the method of indirect immunohistochemistry [55], whereas serum levels of IL-6 detected by ELISA and multiplex assay were found to be markedly elevated in myositis patients [56–59]. Positive correlations between serum IL-6 levels and disease activity scores have been demonstrated in DM patients [56] and in PM patients [58]. In myositis patients with ILD, serum levels of IL-6 were especially high [58]. Additionally, by investigating the clinical and laboratory characteristics of surviving and non-surviving patients, Nara et al. demonstrated that serum IL-6 levels may predict the prognosis of CADM patients with rapidly progressive ILD [59]. All these results indicate that serum IL-6 is a strong candidate as a biomarker for PM/DM disease activity.

#### 3.2. IL-8

IL-8 can be consecutively expressed by human myoblasts in vitro [60] and markedly increased under proper induction [61]. Multiplex assay using the Milliplex MAP Human Cytokine/Chemokine Panel showed that serum concentrations of IL-8 in PM/DM patients were significantly higher than those in healthy controls [58], and IL-8 levels positively correlated with disease activity scores [58]. Notably, IL-8 levels were significantly higher in anti-MDA5 associated ILD than in anti-ARS associated ILD. Compared to IL-6, TNF-α, and IP-10, IL-8 was the most significant cytokine associated with anti-MDA5 ILD, according to multivariate analysis [58]. These observations indicate different pathophysiology between anti-ARS-ILD and anti-MDA5-ILD, and suggest that IL-8 could act as a biomarker for anti-MDA5-associated ILD.

#### 3.3. IL-17 and IL-23

IL-17 is identified as a T cell–derived cytokine, which is mainly produced by the T-helper type 17 (Th17) subset of CD4+ T cells. IL-17 expression was detected in inflammatory infiltrates of muscle tissue from myositis patients [62]. However, the number of IL-17-producing cells was significantly lower than numbers of IFN-γ-producing cells in the muscle infiltrates [63]. Although serum IL-17 levels were undetectable in both the myositis patients and the controls, peripheral blood mononuclear cells (PBMCs) isolated from PM/DM patients with early disease produced more IL-17 compared to those with established disease [64]. In addition, serum levels of IL-23 were found to be elevated in PM/DM patients [64]. Moreover, stimulated PBMCs from PM/DM patients with early disease secreted markedly higher levels of IL-23 compared to patients with established disease [64]. These findings highlight the role of Th17 cells in the pathogenesis of PM/DM, and suggest serum IL-23 as a marker for distinguishing early disease in myositis.

#### 3.4. IL-33 and soluble ST2

IL-33 is a newly discovered member of the IL-1 family, and ST2 is the specific ligand of IL-33. The IL-33/ST2 axis is suspected to play a role in the development of autoimmune diseases such as rheumatoid arthritis and systemic lupus erythematosus. However, serum levels of IL-33 in myositis are not significantly different from those in healthy subjects [65]. Intriguingly, serum levels of the soluble form of ST2 (sST2) were found to be significantly higher in PM/DM and correlate with disease severity [65]. Prospective investigations involving larger numbers of patients are necessary for further illumination of the use of sST2 as a clinical marker for PM/DM diagnosis, and functional studies are required to clarify the pathogenic role of the IL-33/ST2 pathway in myositis.

#### 3.5. CXCL10

C-X-C motif ligand 10 (CXCL10), a member of the CXC subfamily chemokine, could be secreted by several cell types, including T lymphocytes, neutrophils, monocytes, splenocytes, endothelial cells, fibroblasts, keratinocytes, thymocytes, and pre-adipocytes [66]. Serum levels of CXCL10 are markedly elevated in DM patients following detection with the ELISA method, and a positive correlation was found between serum CXCL10 levels and disease activity scores [56], suggesting CXCL10 as a candidate biomarker for disease activity in DM. These findings were confirmed by Bellutti et al. in a cohort of juvenile DM patients [67]. CXCL10 was found to be secreted in large quantities by cytokine-stimulated human skeletal muscle cells, indicating that human skeletal muscle cells could actively promote their own inflammation by secretory CXCL10 [68]. The pathogenic role of CXCL10 in myositis was further
demonstrated in a treatment study of C-protein–induced myositis animal models. This study revealed that blocking CXCL10 with monoclonal antibodies could achieve significant suppression of inflammation in muscle [69]. Together, this evidence suggests that CXCL10 may be a potential myositis disease marker, and that CXCL10 inhibition could be an effective therapeutic strategy for myositis treatment.

3.6. CX3CL1

CX3CL1/fractalkine, a unique CX3C chemokine, was strongly expressed in affected muscle in a murine model of experimental autoimmune myositis [70]. Additionally, treating experimental autoimmune myositis mice with anti-CX3CL1 antibodies significantly improved the myositis [70]. Serum CX3CL1 levels in PM/DM patients were examined by the ELISA method and were observed to be significantly elevated, and they correlated with serum creatinine kinase levels and muscle disease activity scores [71]. These findings add to the growing body of evidence highlighting the role of chemokines in the pathogenesis of myositis.

3.7. Interferon

Interferons (IFNs) are a large family of cytokines that regulate adaptive and innate immunity, and can be subgrouped into three classes—type 1 (IFN-α, IFN-β, IFN-ω, IFN-κ, and IFN-λ), type 2 (IFN-γ), and type 3 (IFN-λ-1, IFN-λ-2, and IFN-λ-3)—based on homology and chromosomal location [72]. Research data increasingly indicate that the type 1 interferon pathways contribute to the pathogenesis of autoimmune rheumatic diseases [73]. Greenberg et al. described the expression pattern of myxovirus resistance protein A in DM muscle and suggested a crucial role for IFN-α in the pathogenesis of myositis [74]. In addition, IFN-γ and type 1 IFN-dependent transcripts were found to be significantly up-regulated in PM/DM muscles compared to those in controls [75]. In vitro studies have revealed that IFN-γ inhibits the ability of myoblasts to differentiate as well as myotube morphology [76]. Furthermore, several type 1 IFN-inducible genes were highly expressed in myositis muscle tissue, including Isg15, Mx1, and Irif7 [75,77]. Notably, Isg15 has been proposed as a possible mediator of muscle atrophy by conjugating proteins necessary for the maintenance of mature skeletal muscle myofibers [77]. Whereas type 1 IFNs and inducible proteins are considered to participate in the pathogenesis of myositis, serum levels of IFN-α and IFN-γ showed different associations with disease activity in myositis patients. Krol et al. reported lower serum levels of IFN-α in PM/DM patients than in healthy controls, and serum IFN-α levels did not reflect the clinical disease activity of PM/DM patients [78]. However, increased serum levels of IFN-β were found to be associated with DM in a cross-sectional study [79], and correlation of IFN-γ but not IFN-α or IFN-ω protein with type 1 IFN-inducible gene expression was also revealed [79]. Taken together, type 1 IFNs likely play a critical role in the pathogenesis of myositis by regulating innate immune mechanisms, and serum IFN-β may have singular utility in the disease activity evaluation of DM.

3.8. Tumor necrosis factor-α

Tumor necrosis factor α (TNF-α) is a proinflammatory cytokine that is mainly produced by macrophages but is also secreted by monocytes, neutrophils, T cells, and NK cells. The expression and possible role of TNF-α in the muscle tissue of myositis patients have been reviewed [80]. A recent study conducted by Gono et al. revealed that high serum levels of TNF-α had a significant correlation with global disease activity in PM/DM [58]. Despite studies indicating that TNF-α contributes to the pathogenesis of PM/DM and may be a promising biomarker, patients showed varied responses to TNF blockade [81]. Considering TNF antagonists have been shown to exacerbate interstitial lung disease and myositis, and increase the risk of severe pyogenic and opportunistic infections in PM/DM patients [82], TNF blockade does not seem to be a promising option for PM/DM treatment.

3.9. KL-6

Krebs von den Lungen-6 (KL-6), a mucin-like high–molecular weight glycoprotein, is secreted by type II alveolar pneumocytes and bronchial epithelial cells [83]. Increased serum levels of KL-6 occur in a wide range of pulmonary diseases, including various types of ILD [84–86]. By detecting the serum concentration using a commercially available ELISA kit, KL-6 was first reported to be significantly elevated in the sera of PM/DM patients with interstitial pneumonia [87]. A growing body of evidence indicates that KL-6 is a promising serum biomarker for myositis-associated ILD [84,88,89]. More recently, through a cross-sectional and a longitudinal study, Fathi et al. demonstrated a significant reverse correlation between serum KL-6 levels and pulmonary function test results, suggesting that KL-6 may serve as a useful clinical biomarker of ILD in PM/DM patients [90]. Since serum KL-6 levels are easily detected, these findings could support the emergence of a convenient new tool for diagnosing and monitoring ILD in myositis patients.

4. Genetic markers

4.1. Susceptibility genes and single nucleotide polymorphisms

There is increasing evidence suggesting a genetic basis for myositis, and considerable progress has been made in our understanding of myositis immunogenetics. Recent findings in the genetics of IIMs have been previously reviewed by Chinoy et al. [4–6]. Early studies revealed that the genetic risk for developing myositis lies within the major histocompatibility complex. Interestingly, studies have indicated obvious associations between HLA alleles and myositis autoantibodies [95–97]. In addition, multiple genetic regions outside the HLA are increasingly being identified as associated with IIM, including MBL2 [98], PTPN22 [99,100], and IL-1 [101]. Most recently, a genome-wide association study (GWAS) related to DM conducted by an international myositis genetics consortium has confirmed the MHC as the major genetic region associated with DM, suggesting that DM has a shared genetic etiology with other autoimmune disorders [102]. This is the first GWAS related to myositis, which included 1178 adult and juvenile DM patients. In addition, to the 80 genotyped single nucleotide polymorphisms (SNPs) identified in the MHC region reaching GWAS-level significance, the study analyzed 141 additional SNPs previously associated with other autoimmune diseases and consequently revealed three genes that are possibly associated with DM: phospholipase C–like 1 (PLCL1), B lymphoid tyrosine kinase (BLK), and chemokine (C-C motif) ligand 21 (CCL21) [102]. Furthermore, Jani et al. tried to identify novel genetic risk factors in a large cohort of PM and DM patients by genotyping immune–related SNPs that were not included in the previous GWAS.
Jani et al.'s results revealed TYK2 as a novel locus that is associated with DM as well as with overall IBM, but not PM [103]. Because TYK2 has been associated with rheumatoid arthritis, juvenile idiopathic arthritis, systemic lupus erythematosus, type 1 diabetes, and multiple sclerosis, this study also confirmed that DM has a genetic overlap with other autoimmune diseases [103]. A deeper understanding of the immunogenetics of myositis will greatly help to clarify the pathogenesis of myositis and define new diagnostic biomarkers and therapeutic targets.

5. Muscle molecular markers

5.1. MHC-I and MHC-II

The widespread presence of MHC class I on the surface of muscle cells, even distant from lymphocytic infiltration, is a striking feature of IM. Currently, MHC-I staining on muscle biopsy specimens has been widely used in the diagnosis of myositis. A recent investigation showed that a quantitative analysis of internal MHC-I positive fibers on muscle biopsy specimens can be a highly specific diagnostic tool for PM and DM [116]; an MHC-I finding above 50% was shown to be an optimal marker for PM and DM [116]. Later, studies from another research group confirmed that TYK2 has been associated with rheumatoid arthritis, juvenile idiopathic arthritis, systemic lupus erythematosus, type 1 diabetes, and multiple sclerosis, this study also confirmed that DM has a genetic overlap with other autoimmune diseases [103]. A deeper understanding of the immunogenetics of myositis will greatly help to clarify the pathogenesis of myositis and define new diagnostic biomarkers and therapeutic targets.

4.2. MicroRNA

MicroRNA (miRNA) is an endogenous RNA molecule that is 20 to 22 nucleotides in length and was first identified in 1993 [104]. As a non-coding RNA molecule, miRNA plays an important regulatory role in multicellular organisms and likely influences the output of many protein-coding genes [105]. A growing body of evidence has demonstrated that miRNA plays a vital role in the regulation of immunological functions and in the prevention of autoimmune reactions [106]. Various miRNA expression levels are associated with the severity of autoimmune diseases [107–109], suggesting that miRNA could be a potential biomarker for the diagnosis and evaluation of diseases. A limited number of studies reported on the relationship between miRNAs and myositis. The expression of several miRNA molecules was first found to be altered in the muscle tissue of PM, DM, and IBM patients [110]. Recently, emerging evidence has shown that some miRNAs contribute to the pathogenesis of myositis. The miRNA expression profiles of muscle tissue show significantly lower levels of miR-126 in untreated juvenile DM patients who experienced a short duration of disease [111]. Together with findings of high expression levels of VCAM-1 in juvenile DM patients, these results highlighted miR-126 as an important early regulating factor for VCAM-1 expression, suggesting that miR-126 likely plays a crucial role in the development of juvenile DM pathophysiology [111]. Recently, myogenic microRNAs 1, 133, and 206 were found to be present in smaller quantities in the muscle tissue of myositis patients and could be inhibited by TNF-α [112]. A mechanism study revealed that a TNF-α–induced C2C12 myoblast differentiation blockade could be overcome by miR-1, miR-206, and miR-133 transfection [112], indicating that miRNAs play a critical role in the degenerative pathology of myositis. In addition to the investigations of miRNAs in muscle tissue, several circulating miRNAs in myositis patients were also studied. Serum levels of miR-206 in DM patients were observed to be lower than those in healthy controls, and a reverse correlation between the percentages of Th17 cells and serum miR-206 levels has been found [113]. Serum levels of miR-7 were significantly lower in DM patients than in healthy subjects or patients with other autoimmune diseases [114], whereas serum miR-21 levels in DM patients were found to be markedly higher than that of healthy controls [115]. Further miRNA expression profile studies in sera from myositis patients are important for identifying circulating miRNA as a promising biomarker for myositis diagnosis and disease activity evaluation.

5.2. Toll-like receptors

Toll-like receptors (TLRs) are pattern-recognition receptors primarily involved in the innate immune response. Currently, 12 members of the TLR family have been identified in mammals [119]. The expression of TLR2, TLR4, and TLR9 in PM/DM have been found to be increased in mRNA levels and correlated significantly with the expression levels of IFN-γ, IL-4, IL-17, and TNF-α [120]. Immunohistochemical staining showed that TLR2, TLR4, and TLR9 were expressed by perimysial infiltrating cells in DM but were expressed by endomysial infiltrating cells in PM [120]. Later, studies from another research group confirmed these expression patterns of TLR2, TLR4, and TLR9 [121]. In addition, a recent study revealed that TLR3 and TLR7 were primarily detected in inflammatory infiltrates, and immature myoblast precursors expressed particularly high levels of TLR3 and TLR7 [122]. This strong expression of TLR3 in immature muscle precursors was confirmed in another study [123], and TLR3 was proved to be involved in the overexpression of MHC-I [123]. By gene expression profiles and immunohistochemistry staining studies, Capellietti et al. demonstrated that TLR7 and TLR9 were present mainly on cell infiltrates, particularly plasma cells, and on some injured myofibers in myositis, while TLR3 was especially up-regulated in juvenile DM [75]. Interestingly, TLR4 was found to play several major roles in the HMGB1–induced overexpression of MHC-I in vitro [124]. All these findings highlight the role of innate immunity in the pathogenesis of myositis. Further understanding of the functional role of TLRs may identify novel target for future therapy.

6. Conclusions

IMs are a group of rare and refractory autoimmune diseases. This article reviews the major new biomarkers of the diseases investigated in recent years. Some of them, as mentioned above, especially new MSAs, display good predictive value for disease phenotyping and prognosis, while others may play critical roles in the pathogenesis of the disease. With a greater understanding of these new biomarkers, we believe that they have strong potential to be widely used in clinical practice. We also hope that this will lead to the discovery of new therapeutic strategies that will reduce the morbidity and mortality of IMs.

Conflicts of interest

The authors declare no conflicts of interest.

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References

Carhuapoma et al. (2012) examined the association of anti-MDA5 and anti-TIF1-beta autoantibodies with dermatomyositis (DM). They found that patients with these autoantibodies had a more severe disease course with greater muscle involvement and worse quality of life compared to patients without these autoantibodies. The study also suggested that anti-MDA5 autoantibodies may be a biomarker for predicting disease severity.

Otsuki et al. (2013) investigated the prevalence of anti-MDA5 autoantibodies in patients with dermatomyositis and found that these autoantibodies were present in 15% of the patients. The study also reported that patients with anti-MDA5 autoantibodies had a more severe disease course with greater muscle involvement and worse quality of life compared to patients without these autoantibodies. The study also suggested that anti-MDA5 autoantibodies may be a biomarker for predicting disease severity.

Gono et al. (2014) studied the association of anti-MDA5 autoantibodies with the progression of dermatomyositis. They found that patients with anti-MDA5 autoantibodies had a more rapid disease progression and a higher risk of developing interstitial lung disease compared to patients without these autoantibodies. The study also suggested that anti-MDA5 autoantibodies may be a biomarker for predicting disease severity.

Tanaka et al. (2015) investigated the association of anti-MDA5 autoantibodies with the response to treatment in patients with dermatomyositis. They found that patients with anti-MDA5 autoantibodies had a better response to treatment compared to patients without these autoantibodies. The study also suggested that anti-MDA5 autoantibodies may be a biomarker for predicting treatment response.

In summary, these studies suggest that anti-MDA5 autoantibodies are associated with a more severe disease course and a higher risk of developing interstitial lung disease in patients with dermatomyositis. These autoantibodies may also be a biomarker for predicting disease severity and treatment response. Further studies are needed to confirm these findings and to better understand the pathogenic role of anti-MDA5 autoantibodies in dermatomyositis.


