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Idiopathic inflammatory myopathies with anti-mitochondrial antibodies: Clinical features and treatment outcomes in a Chinese cohort

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Abstract

Anti-mitochondrial antibodies, the hallmark of primary biliary cirrhosis, have been detected in many patients with idiopathic inflammatory myopathies and these anti-mitochondrial-antibody-associated idiopathic inflammatory myopathies frequently show unique characteristics. We detected anti-mitochondrial antibodies in Chinese idiopathic inflammatory myopathy and summarized the clinical findings of these anti-mitochondrial-antibody-positive patients. Of 136 patients, seven (5.15%) were found to be anti-mitochondrial-antibody-positive. Primary biliary cirrhosis was present in 2 of these 7 patients, chronic disease duration in 2 patients and asymmetrical muscle weakness in 4 patients. The mean disease course was 8.58 months, and the mean creatine kinase level was 2256.53 U/L. Myositis-specific antibodies were found in 3 patients. According to clinical features and muscle histopathological findings, 3 patients were classified as dermatomyositis, 2 as possible polymyositis and 2 as necrotizing autoimmune myopathy. Of the 6 anti-mitochondrial-antibody-positive patients receiving follow-ups of 12–83 months, they all showed marked clinical improvement. Our study indicates that anti-mitochondrial antibodies are relatively rare in Chinese idiopathic inflammatory myopathy patients. These patients generally show various clinical features and have favorable treatment outcomes. Anti-mitochondrial antibody testing may be helpful to confirm the diagnosis of idiopathic inflammatory myopathy, especially in patients with atypical manifestations.

Keywords: Idiopathic inflammatory myopathies; Anti-mitochondrial antibodies; Muscle pathology.

1. Introduction

Idiopathic inflammatory myopathies (IIMs) are a heterogeneous group of autoimmune disorders characterized by proximal muscle weakness and elevated creatine kinase (CK) levels [1]. It has been proposed that patients with specific serum autoantibodies exhibit characteristic clinical features and can be regarded as distinct clinical subsets [2]. Recent studies have found that the prevalence of anti-mitochondrial antibodies (AMAs) in IIMs varied markedly from 0. 6% to 19.5% and these AMA-positive IIM patients generally showed several unique characteristics [3–7].

AMAs are biomarkers of primary biliary cirrhosis (PBC). Although IIMs with AMAs were reported by Sherlock in 1973 [8], large-scale studies have not been carried out until recent years. In Japan, Shimizu and colleagues demonstrated

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that patients with AMAs have a chronic disease course, muscle atrophy, cardiopulmonary involvement and granulomatous inflammation. These conclusions were partly confirmed by Christopher-Stine who revealed that AMA-associated IIMs frequently have chronic skeletal muscle disease and cardiac involvement and can occur in various subgroups. Furthermore, Takeshi Uenaka reported that patients with AMAs have cardiac dysfunctions, longer disease duration, less degree of limb muscle weakness and sparse lymphocytic infiltration than those without AMAs. However, AMA-associated IIMs did not seem to be associated with cardiac involvement, muscular atrophy or a particular pathological feature in a European cohort as reported by Benveniste [3–6].

To date, no study has evaluated the prevalence, clinical features and treatment outcomes of AMA-associated IIMs in a Chinese patient cohort. Thus, we explored the prevalence of AMAs among 136 IIM patients and summarized the clinical, serological, histopathological characteristics and treatment outcomes of 7 AMA-positive patients.

2. Materials and methods

2.1. Participants

Clinical data and serum samples were collected from 136 patients with IIM in the Department of Neurology at Qilu Hospital from April 2005 to December 2017. The diagnosis and classification of IIM was based on the criteria proposed by the European Neuromuscular Centre (ENMC) [9]. We reviewed their clinical manifestations, laboratory findings, histopathological characteristics, treatment regimens and outcomes. This study was approved by the Qilu hospital (Qingdao) institutional ethics committee. Informed consent was obtained from all the patients and control individuals.

2.2. Clinical assessment

"Chronic disease duration" was defined as a duration from disease onset to first examination over 12 months [10,11]. Muscle strength was evaluated by the ordinal six-point 0–5 of manual muscle testing (MMT), and severe weakness was defined as no more than grade 3 in the weakest muscle [12]. PBC was diagnosed according to the criteria proposed by the American Association for the Study of Liver Disease [13].

2.3. Detection of myositis-specific antibodies (MSAs), myositis-associated autoantibodies (MAAs) and AMAs

Patient serum was stored at −80 °C. MSAs including anti-signal recognition particle (SRP), anti-EJ, anti-Jo-1, anti-OJ, anti-PL-12, anti-PL-7, anti-Mi-2, anti-melanoma differentiation-associated gene 5 (MDA5), anti-transcriptional intermediary factor 1γ (TIF1γ), anti-nuclear matrix protein 2 (NXP2), anti-small ubiquitin-like modifier activating enzyme (SAE) and MAAs including anti-KU, anti-PM-Scl 75, anti-PM-Scl 100, anti-RO-52, were detected by Euroimmun immunoblots based on standard methods (Euroline Myositis Profile 3 immunoline-blot; Euroimmun) in 136 patients with IIM. Anti-3-hydroxy-3-methylglutaryl-CoA reductase (HMGCR) autoantibodies were measured by enzyme-linked immunosorbent assay test with recombinant HMGCR antigen (Sigma-Aldrich, St. Louis, MO, USA) in 136 patients with IIM and in 24 healthy controls. AMAs were tested by line immunoassay according to the manufacturer's protocol (Autoimmune Liver Diseases Antibodies Profile Line Immuno Assay, HOB, China) in 136 IIM patients. Five patients were re-tested by this method after successful treatment. Moreover, eleven samples from all 7 AMA-positive patients detected by line immunoassay, including 6 samples before or shortly after treatment and 5 samples after successful treatment, were then tested by indirect immunofluorescence (IIF) using rat kidney and stomach (Fluoro aid-1 test, 4250CN, Medical & Biological Laboratories, Japan) (Supplement 1).

2.4. Muscle biopsy

Muscle biopsies were performed before corticosteroid treatment. Serial frozen sections were stained with hematoxylin and eosin (HE), modified Gomori trichrome (MGT) and nicotinamide adenine dinucleotide (NADH). Anti-CD3 mouse monoclonal antibody (clone LN10; Zhongshan Golden Brige Biotechnology, Beijing, China), anti-CD8 rabbit monoclonal antibody (clone SP16; Zhongshan Golden Brige Biotechnology, Beijing, China), anti-major histocompatibility complex class I (MHC-I) rabbit monoclonal antibody (clone EP1395Y; Abcam, London, Britain), anti-major histocompatibility complex class II (MHC-II) mouse monoclonal antibody (clone CR3/43; Dako, Glostrup, Denmark) and anti-membrane attack complex (MAC) mouse monoclonal antibody (clone aE11; Dako, Glostrup, Denmark) were stained in sections of all 136 patients. The subgroups of IIM, such as polymyositis (PM), dermatomyositis (DM), non-specific myositis (NSM) and necrotizing autoimmune myopathy (NAM), were classified by the ENMC criteria [9,14].

2.5. Treatment outcome measures

According to the definition of improvement made by the International Myositis Assessment and Clinical Studies Group, improvement was defined as three of any six core set measures improved $\geq$20%, with no more than two measures worsening by $\geq$25% (which cannot be MMT) [15,16]. Treatment outcomes were graded as no improvement, mild improvement (1 grade in at least one muscle group, persistently requiring assistance in daily activities), moderate improvement (>1 grade in multiple muscle groups, occasionally requiring assistance in daily activities), marked improvement (only mild weakness without functional impairment), and return to baseline (no symptoms or signs of muscle weakness) [17]. A favorable outcome was defined as marked improvement or return to baseline [17].

Table 1
Clinical features of 7 AMA-associated IIM patients.

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7
Detection methods							
Line immunoassay	+	+	+	+	+	+	+
IIF	+	+	+	+	–	+	+
Classification	DM	NAM	DM	PM	DM	PM	NAM
Demographic data							
Sex	F	F	M	F	F	M	M
Age (y)	50	41	57	53	70	57	55
Disease duration (mo)	4	1	12	18	4	24	5
Signs and symptoms							
Proximal limb weakness	+	+	+	+	+	+	+
Neck weakness	+	+	–	+	+	–	–
Dysphagia	+	+	–	+	+	+	–
Distal muscle weakness	+	+	–	+	+	–	–
Asymmetrical limb weakness	–	+	–	+	+	–	+
Severe limbs weakness	+	+	–	+	+	–	–
Atrophy	–	+	–	–	+	+	–
Myalgia	+	–	–	+	+	+	–
Dysarthria	–	+	–	+	+	–	–
Dyspnea	–	+	–	–	–	–	–
Skin rash	+	–	+	–	+	–	–
PBC	–	+	+	–	–	–	–
Cardiac involvement	–	+	–	–	–	+	–
Hepatic hemangioma	–	+	–	–	–	–	+
Possible malignancy	–	–	–	–	+	–	–
Laboratory data							
CK (U/L)	190	102	2354	1740	1337	6352	4200
MSA	Anti- NXP2 +++	–	Anti- TIF1γ +	–	Anti- NXP2 +++	–	–

Abbreviations: AMA, anti-mitochondrial antibody; IIM, idiopathic inflammatory myopathy; IIF, indirect immunofluorescence; DM, dermatomyositis; PM, polymyositis; NAM, necrotizing autoimmune myopathy; F, female; M, male; PBC, primary biliary cirrhosis; CK, creatine kinase; MSA, myositis-specific antibodies; anti-NXP2, anti-nuclear matrix protein 2; anti-TIF1γ, anti-transcriptional intermediary factor 1γ.

2.6. Statistical analysis

Categorical variables were analyzed by Fisher's exact test as a predicted frequency <5 and the significant p level was corrected by the Bonferroni method since multiple comparisons were made. The calculations were performed using the SPSS 20.0 statistical analysis software.

3. Results

3.1. Frequency of AMAs in IIM patients

AMAs were detected in 7 (5.15%) out of 136 IIM patients by line immunoassay. Among them, 3 patients were classified as DM, 2 patients as possible PM including one with CD8+ T cells surrounding non-necrotic fibers and the other with ubiquitous MHC-I expression, and 2 patients as NAM. All 7 patients except Patient 5 were AMA-positive by confirmatory IIF (Table 1). Two patients (one with NAM and one with DM) were associated with PBC.

3.2. Clinical features

The clinical features of the 7 patients with AMA are summarized in Table 1. There were 3 males and 4 females with ages at onset ranging from 41 to 70 years (mean ± SD, 54.71 ± 8.73 years). The duration from disease onset to the first visit varied from 1 to 24 months (mean ± SD, 8.58 ± 9.71 months) and chronic disease duration occurred in 2 patients without PBC. Indeed, asymmetrical limb weakness, which is infrequent in IIM, was seen in 4 patients including one with PBC. In addition, four patients experienced neck weakness, and 4 patients had distal muscle weakness. Severe limbs weakness was observed in 4 patients, and muscle atrophy was found in 3 patients. Dysphagia was seen in 5 patients, dysarthria in three patients, myalgia in four patients and dyspnea in one patient who needed ventilatory support.

With regard to extramuscular manifestations, a periorbital heliotrope rash was found in all three DM patients. Cardiac muscle involvement was seen in two cases (one with PBC), including one with atrial premature beat and the other with complete right bundle branch block. One patient had a suspected malignancy as positron emission computed tomography revealed that fluorodeoxyglucose was highly absorbed by multiple lymph nodes in the right axillary area and under the chest muscle of the right anterior chest wall; however, this patient declined to undergo a biopsy. Furthermore, two patients had hepatic hemangioma (one of them was associated with PBC). No rheumatic disease was found in these 7 patients.

3.3. Laboratory results

Serum CK levels in the 7 patients with AMAs ranged from 102 to 6352 IU/L (mean ± SD, 2256.53 ± 2325.00 U/L), and

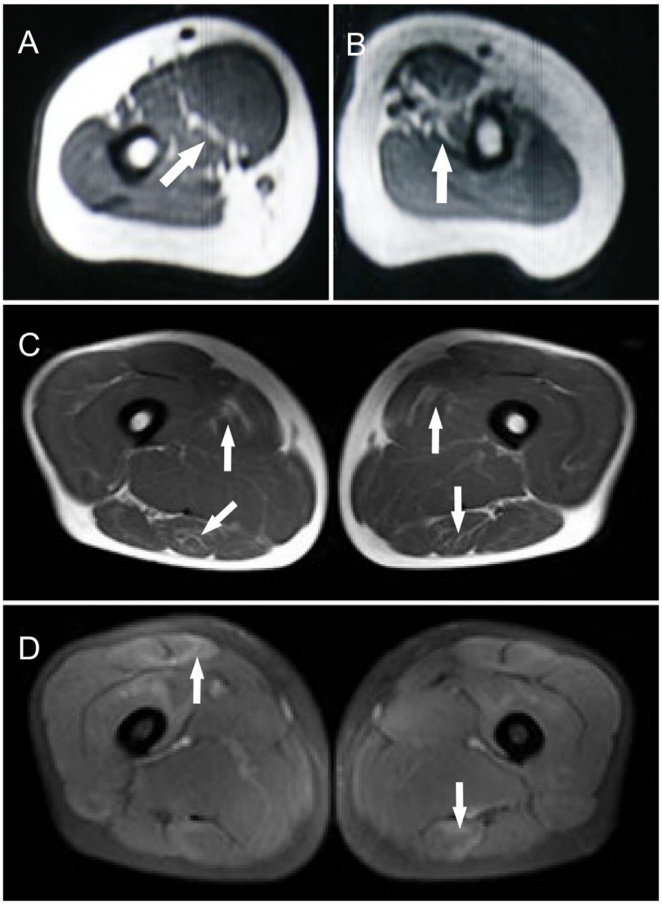

Fig. 1. MRI changes in AMA-associated IIMs. (A and B) T1-weighted images showed asymmetrical fatty infiltration and atrophy in the arms in Patient 4 (white arrows). (C) T1-weighted images showed focally fatty infiltration in the muscles of the thigh in Patient 7 (white arrows). (D) A STIR image revealed mild focal edema in the muscles of the thigh in Patient 7 (white arrows). Abbreviations: MRI, magnetic resonance imaging; AMA, anti-mitochondrial antibody; IIM, idiopathic inflammatory myopathy; STIR, short-tau inversion recovery.

serum CK levels were 102 U/L and 2354 U/L respectively in the two AMA-positive patients with PBC. Anti-NXP2 antibody was strongly positive (+++) in 2 patients (both without PBC), and anti-TIF1γ antibody was mildly positive (+) in 1 patient with PBC. Four patients had anti-RO-52 antibodies, while other MAAs were not found in 7 AMA-positive patients. Muscle CT was performed in one patient with PBC and showed remarkable atrophy in the posterior compartment of the thigh. Magnetic resonance imaging (MRI) was carried out in three patients (one of the upper limbs and two of the lower limbs). MRI showed asymmetrical fatty infiltration and atrophy in the biceps brachii muscles and focal edema and fatty infiltration in the thighs (Fig. 1).

3.4. Histopathological findings of muscle biopsies

The seven patients with AMA were classified as 3 DM, 2 PM and 2 NAM (Fig. 2). Considering that of the 129 AMA-negative patients, 70 were diagnosed as DM, 24 as PM, 17 as NSM and 18 as NAM, AMAs were not associated with a specific subgroup ($p = 0.386$). Cytoplasmic body and intermyofibrill network disorder in non-necrotic fibers were found in Patient 5. Sarcoplasmic mass and lobulated fibers were shown in Patient 4 (Fig. 3). Deposition of MAC was found in the sarcolemma of non-necrotic muscle fibers in 1 patient (without PBC) and in the capillaries in 4 patients (one patient with PBC and three patients without PBC). MHC-II was diffusely expressed in the sarcolemma of muscle fibers in 5 patients (two patients with PBC) and focally expressed in 1 patient (without PBC) (Fig. 4).

3.5. Treatment and outcomes

The detailed drug therapy and treatment outcomes of the 7 AMA-positive patients are summarized in Table 2. All patients were initially treated with oral prednisone (1 mg/kg/day), and three required additional immunotherapies. Specifically, one was treated with intravenous immunoglobulin (with PBC), one with intravenous cyclophosphamide (without PBC), and another with methotrexate (MTX) which was then replaced by azathioprine (with PBC). One patient died of possible cancer metastasis 2 months after treatment. All 6 remaining patients had follow-up for more than 12 months (range, 12.00–82.79 months; median, 30.26 months). The median time of improvement was 2 months (range, 1–4 months) after initial treatment. Two patients suffered from relapse during tapering or discontinuation of drugs. At the time of the last follow-up, all six patients demonstrated marked improvement, and 2 patients were drug free (one with PBC). CK levels declined to less than 400 U/L in all 6 patients and to normality in two patients (both without PBC). AMAs detected by both line immunoassay and IIF were still present after successful treatment in 4 of 5 patients.

To further explore the characteristics of AMA-associated IIMs, we summarized the clinical features of our patients and previously reported patients in Table 3. There was considerable variability in the prevalence of AMAs among patients with IIM and in the clinical and pathological features of these AMA-positive patients.

4. Discussion

This is the first study to evaluate the prevalence of AMAs in a large sample of Chinese IIM patients and to comprehensively analyze the clinical features and treatment outcomes of these AMA-positive patients. We find that AMAs are relatively rare in Chinese IIM patients. These AMA-positive patients could have various clinical features and favorable treatment outcomes. In fact, atypical IIM features that may be misdiagnosed as muscle dystrophy (MD), such as chronic progression and asymmetrical muscle weakness, occurred in some of our AMA-positive patients. Considering that AMAs have not yet been reported in other muscular disorders, we recommend AMA testing to confirm the diagnosis of IIM, especially in patients with atypical IIM features.

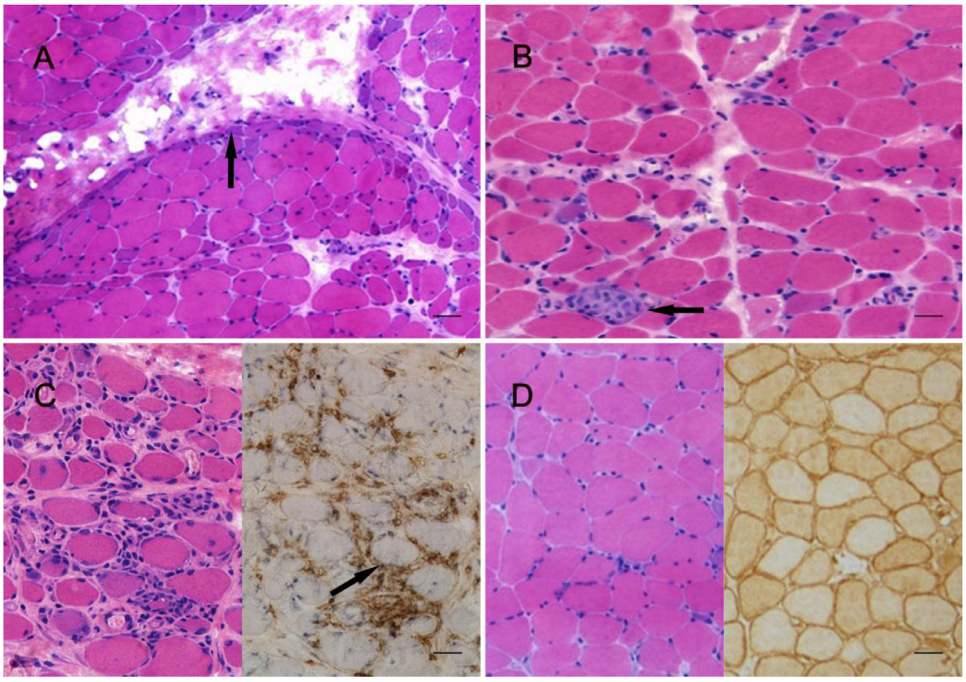

Fig. 2. Histological subgroups of AMA-associated IIMs. (A) Dermatomyositis with perifascicular atrophy in Patient 1 (black arrow). (B) Necrotizing autoimmune myopathy in Patient 2 (black arrow indicating necrotic fiber). (C) Possible polymyositis with endomysial CD8+ T cells infiltration (black arrow) in Patient 4 (HE and CD8 stain). (D) Possible polymyositis with ubiquitous MHC-I expression in Patient 6 (HE and MHC-I stain). Bar: 50 μm in (A–D). Abbreviations: AMA, anti-mitochondrial antibody; IIM, idiopathic inflammatory myopathy; HE, hematoxylin and eosin; MHC-I, major histocompatibility complex class I.

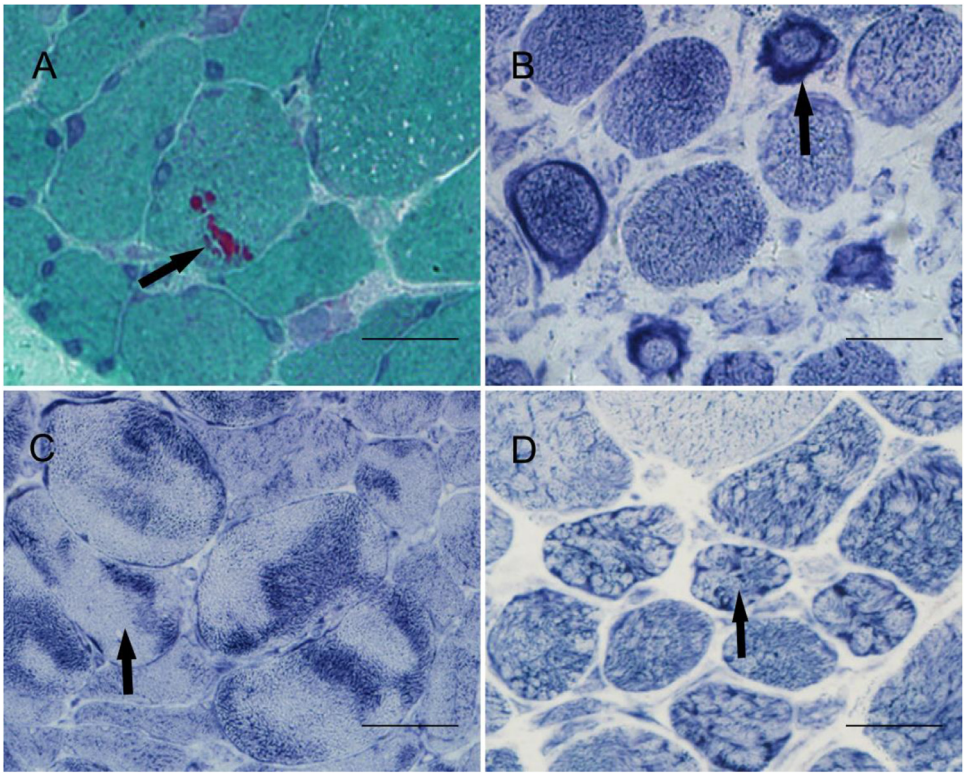

Fig. 3. Histochemical staining in AMA-associated IIMs. (A) Cytoplasmic body (black arrow) on MGT staining in Patient 5. (B) Sarcoplasmic mass (black arrow) on NADH staining in Patient 4. (C) Intermyofibrill network disorder (black arrow) on NADH staining in Patient 5. (D) Lobulated fibers (black arrow) on NADH staining in Patient 4. Bar: 50 μm in (A–D). Abbreviations: AMA, anti-mitochondrial antibody; IIM, idiopathic inflammatory myopathy; MGT, modified Gomori trichrome; NADH, nicotinamide adenine dinucleotide.

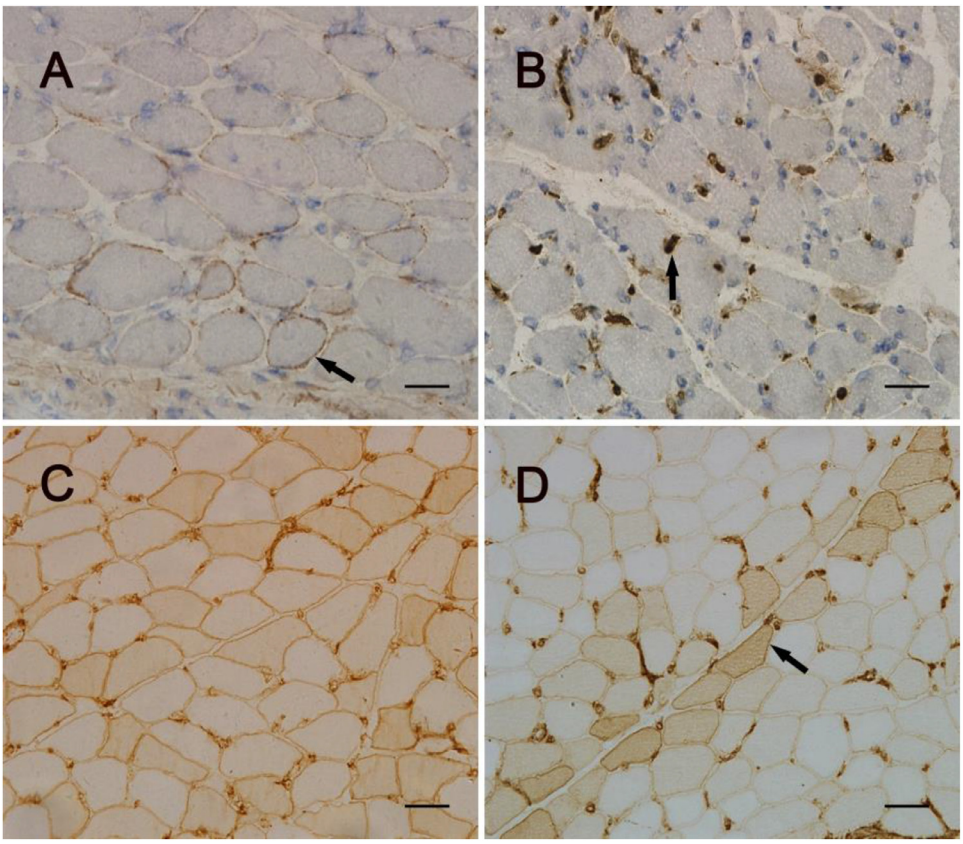

Fig. 4. Immunohistochemical staining in AMA-associated IIMs. (A) Deposition of MAC in the sarcolemma of non-necrotic fibers (black arrow) in Patient 4. (B) Deposition of MAC in the endomysial capillaries (black arrow) in Patient 5. (C) Ubiquitous MHC-II expression in Patient 3. (D) Focal MHC-II expression (black arrow) in Patient 6. Bar: 50 μm in (A–D). Abbreviations: AMA, anti-mitochondrial antibody; IIM, idiopathic inflammatory myopathy; MAC, membrane attack complex; MHC-II, major histocompatibility complex class II.

Table 2
Therapeutic outcomes of 7 AMA-associated IIM patients.

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7
Drugs							
Prednisone	+	+	+	+	+	+	+
Additional immunotherapies	–	IVIG	MTX, AZA	–	CTX	–	–
Duration of follow-up (mo)	82.79	68.57	34.15	26.37	2.00	23.34	12.00
Improvement after therapy (mo)	1	2	4	2	No	1	2
Relapse	–	+	+	–	–	–	–
Drugs at last follow-up							
Prednisone	–	–	10 mg/qd	10 mg/qd	50 mg/qd	5 mg/qd	5 mg/qd
Additional immunotherapies	–	–	AZA 150 mg/qd	–	CTX 0.8 g/qw	–	–
CK at last follow-up (U/L)	Normal	373	390	283	NI	Normal	230
Outcomes	MI	MI	MI	MI	Death[a]	MI	MI

[a] This patient died 2 months after treatment.Abbreviations: AMA, anti-mitochondrial antibody; IIM, idiopathic inflammatory myopathy; IVIG, intravenous immunoglobulin; MTX, methotrexate; AZA, azathioprine; CTX, cyclophosphamide; CK, creatine kinase; MI, marked improvement.

In our study, line immunoassay and IIF showed consistent results in 10 of 11 samples (including samples collected from 4 patients after long-term treatment). Specifically, a sample from one patient who was AMA-positive by line immunoassay was not confirmed by IIF. In fact, we re-tested this sample by immunoassay and still found a positive result. Since the sensitivity of AMAs measured by IIF ranged largely from 43.8% to 100% [18], we regarded this patient as a possible case of AMA-associated IIM.

In our study, the prevalence of AMAs in IIM patients was 5.15%, which is similar to that reported in France (7.80%) but quite higher than that reported in America (0.6%) and lower than that reported in Japan (11.3–19.5%) [3-6]. Although the Bohan and Peter inclusion criteria of low specificity used in the American research may partly explain the reason for our relatively higher AMA prevalence than that in America, there is still conspicuous heterogeneity in the prevalence of AMAs [19].

Table 3
Summary of the clinical features of IIM patients with serum AMAs.

Countries reported	Japan [3]	France [4]	America [5]	Japan [6]	Our study
Frequency of AMA	11.3% (24/212)	7.8% (11/141)	0. 6% (7/1180)	19.5% (8/41)	5.1% (7/136)
Classification (%)	NI	DM 1 (9.1) PM 5 (45.4) NAM 2 (18.2) ASS 2 (18.2)	DM 1 (14.3) PM 1 (14.3) NSM 1 (14.3) NAM 4 (57.1)	DM 1 (12.5) PM 7 (87.5)	DM 3 (42.9) PM 2 (28.6) NAM 2 (28.6)
Demographic data					
Age (y)	54 (32–86)	45.6 (22.7-76.7)	55 (46–64)	NI	55 (41–70)
Average disease duration (mo)	20 (1–60)	NI	78 (3.6–120)	76.8	5 (1–24)
Chronic progression (%)	13 (54.2)	NI	6 (85.7)	NI	2 (28.6)
Signs and symptoms					
Axial involvement (%)	3 (12.5)	3 (42.9)	NI	NI	0 (0.0)
Dysphagia (%)	4 (19.0)	3 (27.3)	4 (57.2)	NI	5 (71.4)
Atrophy (%)	13 (54.2)	3 (33.3)	6 (85.7)	NI	3 (42.9)
PBC (%)	7 (29.2)	3 (27.3)	1 (14.3)	6 (75.0)	2 (28.6)
Cardiac involvement (%)	8 (33.3)	4 (36.7)	5 (71.4)	5 (62.5)	2 (28.6)
Lung involvement (%)	6 (25.0)	3 (27.3)	0 (0.0)	5 (62.5)	1 (14.3)
Other autoimmune disease (%)	7 (29.2)	5 (45.5)	3 (42.9)	NI	0 (0.0)
Laboratory data					
CK (U/L)	2322 (232– 15,132)	1500 (481–8100)	2000 (846–3024)	1050 ± 1137	1740 (190–6352)
MSA (%)	Anti-Jo11 (4.2)	Anti-SRP 1 (9.1) Anti-HMGCR 1 (9.1) Anti-PL-7 1 (9.1) Anti-Jo1 1 (9.1)	Anti-HMGCR 1 (14.3)	None	Anti-NXP2 2 (28.6) Anti-TIF1γ 1 (14.3)
Pathologic features					
Inflammation (%)	23 (95.8)	NI	3 (42.9)	1 (12.5)	3 (42.9)
Perifascicular atrophy (%)	0 (0.0)	NI	1 (14.2)	NI	1 (14.2)

Abbreviations: IIM, idiopathic inflammatory myopathy; AMA, anti-mitochondrial antibody; NI, no information; DM, dermatomyositis; PM, polymyositis; NAM, necrotizing autoimmune myopathy; ASS, anti-synthetase syndrome; NSM, non-specific myositis; PBC, primary biliary cirrhosis; CK, creatine kinase; MSA, myositis-specific antibodies; anti-SRP, anti-signal recognition particle; anti-HMGCR, anti-3–hydroxy–3-methylglutaryl-CoA reductase; anti-NXP2, anti-nuclear matrix protein 2; anti-TIF1γ, anti-transcriptional intermediary factor 1γ.

Indeed, we found that the clinical features in patients with AMAs showed great variability. Firstly, although AMAs only occurred in adult IIM patients, the age at onset ranged from 23 years to 86 years and the mean age at onset ranged from the fourth decade to the seventh decade [3–5,7,8,20–26]. Besides, the average disease duration varied from 10 months (in our study) to 6.5 years, and the prevalence of chronic disease duration varied from approximately 30% (in our study) to 85% [3,5,6]. In fact, although AMAs are regarded as the serological hallmarks of PBC, they are not always associated with PBC in IIM patients. Specifically, the frequency of PBC ranged largely from 15–75% [3–6]. The frequency of cardiac involvement is relatively higher in AMA-associated IIM [3,5,6,11,17,27]. This may be partly due to the presence of PBC because it is associated with increased cardiovascular disease risk [3,28–30]. Thus, patients with AMAs should be followed up carefully for cardiac complications. In fact, the frequency of cardiac involvement also had a certain variability, ranging from approximately 30% to 70% [3–6]. Furthermore, dysphagia, muscle atrophy, skin rash and restrictive ventilatory impairment also varied markedly in AMA-positive patients [3–6]. Indeed, lung involvement was not easy to evaluate as it was not clearly and consistently defined. Specifically, some have reported the frequency of interstitial lung disease and others have studied the frequency of restrictive ventilatory impairment [3–6]. In our cases, other autoimmune diseases such as systemic sclerosis, systemic lupus erythematosus or rheumatoid arthritis were not found, though they have been described in patients with AMAs and may even account for about 30–40% of AMA-associated patients [3–5,31–36]. The abovementioned clinical manifestations may indicate that AMA-positive IIMs are a subgroup of IIMs with a certain variability.

Indeed, several unique clinical features which are similar to those seen in MD, such as chronic progression and asymmetrical muscle weakness, were not uncommon in AMA-positive IIM patients both in our study and in previous reports [3,5,6]. Other unique clinical characteristics that are rarely seen in IIM such as scapular winging and axial muscle involvement including lordotic posture, though not found in our patients, have been reported in previous studies [3–6]. Since these features often occur in MD, Pompe or other muscle diseases in which AMAs have not yet been described, AMA testing could be considerably useful in the differential diagnosis between atypical IIM and other muscle diseases. Besides, hepatic hemangioma that has never been described in AMA-positive IIM patients was found in two of our patients. Whether the coexistence of these two diseases is incidental or implies a closer relationship between them remains unclear.

With regard to the laboratory examinations of patients with AMAs, the mean CK level was about 2000 U/L both in our study and in previous reports [3–6]. The positive rate of many

types of MSA were relatively low, despite the testing panels of MSA being different from those used in previous reports [3–5]. In fact, all 4 AMA-associated non-DM-IIM patients in our study did not have positivity for any MSAs. Since circulating AMAs have not yet been reported in other muscular diseases expect IIM, AMA detection could be regarded as a useful method to confirm the diagnosis of IIM, especially in patients without MSA.

Indeed, AMAs can be observed in various subgroups of IIMs in our research, indicating that AMAs could not be used as biomarkers for a specific subgroup. Regarding specific histopathological features, the frequency of perifascicular atrophy was generally low, while the frequency of inflammation varied largely from 12.5% to 95.8% [3–7]. The variety of clinical and histopathological features found in patients with AMAs could indicate that AMA-associated IIMs are a subgroup of IIMs with various characteristics.

AMA-positive patients were generally treated with prednisone or prednisone with other immunotherapies. Although the frequency of patients receiving other immunotherapies varied considerably from approximately 10% to 90%, all alive patients generally had a good response to the therapy as indicated by the improvement in MMT and/or the decrease in CK levels [3,4,6]. However, the titer of AMA did not decrease in a majority of the patients after successful treatment, which may be partly due to the nature of AMAs [3]. Indeed, AMA titers do not change over time and are not correlated with disease activity or progression in PBC patients either [3,37,38]. Therefore, it is not advisable to use AMAs to modulate disease activity. As the treatment and outcomes were not comprehensively analyzed in previous studies, more studies are needed to confirm our findings.

We are aware of several limitations of the present study. Firstly, the number of patients with AMAs is small, thus the clinical features and treatment outcomes of AMA-positive patients need to be confirmed in further studies, especially in comparative studies. Secondly, some serum samples used for detecting AMAs were obtained during treatment or post-treatment, which limits the accuracy of the prevalence of AMAs. Nevertheless, this is a minor restriction since AMAs did not become negative after treatment in the majority of patients with our testing methods. Thirdly, the exact prevalence of AMAs in IIM was uncertain as the comparison of the sensitivity and specificity between our two testing methods was not reported. However, this is also a minor limitation because our two methods showed consistent results in most samples and the features of AMA-associated IIM that various clinical manifestations and favorable treatment outcomes were unchanged regardless of the detection methods. Indeed, AMAs might need to be tested in multiple methods to obtain a comprehensive result, and the optimal method for detecting AMAs needs to be explored.

5. Conclusions

AMAs are relatively rare in Chinese patients with IIM. These AMA-positive patients generally show various clinical and histopathological features and favorable treatment outcomes. In addition, we suggest that AMA testing be performed to aid in the diagnosis of IIM, especially in patients with atypical IIM manifestations. Given that our present study is a retrospective review of a relatively small number of AMA-associated IIM patients, future studies involving a large cohort of patients are necessary to support our conclusions.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.nmd.2018.11.004.

References

[1] Dalakas MC. Inflammatory muscle diseases. N Engl J Med 2015;372(18):1734–47.
[2] Suzuki S, Uruha A, Suzuki N, Nishino I. Integrated diagnosis project for inflammatory myopathies: an association between autoantibodies and muscle pathology. Autoimmun Rev 2017.
[3] Maeda MH, Tsuji S, Shimizu J. Inflammatory myopathies associated with anti-mitochondrial antibodies. Brain 2012;135(Pt 6):1767–77.
[4] Mauhin W, Mariampillai K, Allenbach Y, Charuel JL, Musset L, Benveniste O. Anti-mitochondrial antibodies are not a hallmark of severity in idiopathic inflammatory myopathies. Jt Bone Spine 2018;85(3):375–6.
[5] Albayda J, Khan A, Casciola-Rosen L, Corse AM, Paik JJ, Christopher-Stine L. Inflammatory myopathy associated with anti-mitochondrial antibodies: a distinct phenotype with cardiac involvement. Semin Arthritis Rheum 2018;47(4):552–6.
[6] Uenaka T, Kowa H, Ohtsuka Y, Seki T, Sekiguchi K, Kanda F, et al. Less limb muscle involvement in myositis patients with anti-mitochondrial antibodies. Eur Neurol 2017;78(5–6):290–5.
[7] Allenbach Y, Mammen AL, Benveniste O, Stenzel W. Immune-mediated necrotizing myopathies working G. 224th ENMC international workshop: clinico-sero-pathological classification of immune-mediated necrotizing myopathies Zandvoort, The Netherlands, 14–16 October 2016. Neuromuscul Disord 2018;28(1):87–99.
[8] Sherlock S, Scheuer PJ. The presentation and diagnosis of 100 patients with primary biliary cirrhosis. N Engl J Med 1973;289(13):674–8.
[9] Hoogendijk JE, Amato AA, Lecky BR, Choy EH, Lundberg IE, Rose MR, et al. 119th ENMC international workshop: trial design in adult idiopathic inflammatory myopathies, with the exception of inclusion body myositis, 10–12 October 2003, Naarden, The Netherlands. Neuromuscul Disord 2004;14(5):337–45.

[10] Watanabe Y, Uruha A, Suzuki S, Nakahara J, Hamanaka K, Takayama K, et al. Clinical features and prognosis in anti-SRP and anti-HMGCR necrotising myopathy. J Neurol Neurosurg Psychiatry 2016.
[11] Suzuki S, Yonekawa T, Kuwana M, Hayashi YK, Okazaki Y, Kawaguchi Y, et al. Clinical and histological findings associated with autoantibodies detected by RNA immunoprecipitation in inflammatory myopathies. J Neuroimmunol 2014;274(1-2):202–8.
[12] Suzuki S, Hayashi YK, Kuwana M, Tsuburaya R, Suzuki N, Nishino I. Myopathy associated with antibodies to signal recognition particle: disease progression and neurological outcome. Arch Neurol 2012;69(6):728–32.
[13] Lindor KD, Gershwin ME, Poupon R, Kaplan M, Bergasa NV, Heathcote EJ, et al. Primary biliary cirrhosis. Hepatology 2009;50(1):291–308.
[14] De Bleecker JL, De Paepe B, Aronica E, de Visser M, Amato A, et al., Group EMMBS 205th ENMC international workshop: pathology diagnosis of idiopathic inflammatory myopathies part II 28-30 March 2014, Naarden, The Netherlands. Neuromuscul Disord 2015;25(3):268–72.
[15] Miller FW. New approaches to the assessment and treatment of the idiopathic inflammatory myopathies. Ann Rheum Dis 2012;71(Suppl 2):i82–5.
[16] Rider LG, Giannini EH, Brunner HI, Ruperto N, James-Newton L, Reed AM, et al. International consensus on preliminary definitions of improvement in adult and juvenile myositis. Arthritis Rheum 2004;50(7):2281–90.
[17] Kassardjian CD, Lennon VA, Alfugham NB, Mahler M, Milone M. Clinical features and treatment outcomes of necrotizing autoimmune myopathy. JAMA Neurol 2015;72(9):996–1003.
[18] Hu S, Zhao F, Wang Q, Chen WX. The accuracy of the anti-mitochondrial antibody and the M2 subtype test for diagnosis of primary biliary cirrhosis: a meta-analysis. Clin Chem Lab Med 2014;52(11):1533–42.
[19] Linklater H, Pipitone N, Rose MR, Norwood F, Campbell R, Salvarani C, et al. Classifying idiopathic inflammatory myopathies: comparing the performance of six existing criteria. Clin Exp Rheumatol 2013;31(5):767–9.
[20] Tanaka T, Suzuki S, Nishino I, Hamaguchi Y, Fujimoto T. What is the third serological marker associated with immune-mediated necrotizing myopathy? Scand J Rheumatol 2017:1–2.
[21] Shichijo K, Mitsui T, Kunishige M, Kuroda Y, Masuda K, Matsumoto T. Involvement of mitochondria in myasthenia gravis complicated with dermatomyositis and rheumatoid arthritis: a case report. Acta Neuropathol 2005;109(5):539–42.
[22] Watt FE, James OF, Jones DE. Patterns of autoimmunity in primary biliary cirrhosis patients and their families: a population-based cohort study. QJM 2004;97(7):397–406.
[23] Uenaka T, Kowa H, Sekiguchi K, Nagata K, Ohtsuka Y, Kanda F, et al. Myositis with antimitochondrial antibodies diagnosed by rectus abdominis muscle biopsy. Muscle Nerve 2013;47(5):766–8.
[24] Pamfil C, Candrea E, Berki E, Popov HI, Radu PI, Rednic S. Primary biliary cirrhosis–autoimmune hepatitis overlap syndrome associated with dermatomyositis, autoimmune thyroiditis and antiphospholipid syndrome. J Gastrointest Liver Dis 2015;24(1):101–4.
[25] Matsumoto K, Tanaka H, Yamana S, Kaneko A, Tsuji T, Ryo K, et al. Successful steroid therapy for heart failure due to myocarditis associated with primary biliary cirrhosis. Can J Cardiol. 2012;28(4) 515 e3–6.
[26] Yamanaka T, Fukatsu T, Ichinohe Y, Hirata Y. Antimitochondrial antibodies-positive myositis accompanied by cardiac involvement. BMJ Case Rep 2017;2017.
[27] Kao AH, Lacomis D, Lucas M, Fertig N, Oddis CV. Anti-signal recognition particle autoantibody in patients with and patients without idiopathic inflammatory myopathy. Arthritis Rheum 2004;50(1):209–15.
[28] Selmi C, Bowlus CL, Gershwin ME, Coppel RL. Primary biliary cirrhosis. Lancet 2011;377(9777):1600–9.
[29] Jackson H, Solaymani-Dodaran M, Card TR, Aithal GP, Logan R, West J. Influence of ursodeoxycholic acid on the mortality and malignancy associated with primary biliary cirrhosis: a population-based cohort study. Hepatology 2007;46(4):1131–7.
[30] Jones DE, Hollingsworth K, Fattakhova G, MacGowan G, Taylor R, Blamire A, et al. Impaired cardiovascular function in primary biliary cirrhosis. Am J Physiol Gastrointest Liver Physiol 2010;298(5):G764–73.
[31] Miyachi K, Hankins R, Ihara M, Miyamoto A, Okano T, Iwai M, et al. A case of limited cutaneous systemic sclerosis developing anti-mitochondria antibody positive primary biliary cirrhosis after acute myocardial infarction. Clin Rheumatol 2007;26(9):1571–4.
[32] Li CH, Xu PS, Wang CY, Zou GL. Increased serum aminotransferases associated with anti-mitochondrial antibodies in systemic lupus erythematosus patients with autoimmune liver disease. Clin Chim Acta 2006;365(1-2):135–42.
[33] Fujimoto M, Sato S, Ihn H, Tamaki T, Kikuchi K, Soma Y, et al. Autoantibodies to mitochondrial 2-oxo-acid dehydrogenase complexes in localized scleroderma. Clin Exp Immunol 1996;105(2):297–301.
[34] Wang L, Zhang FC, Chen H, Zhang X, Xu D, Li YZ, et al. Connective tissue diseases in primary biliary cirrhosis: a population-based cohort study. World J Gastroenterol 2013;19(31):5131–7.
[35] Ramos-Casals M, Garcia-Carrasco M, Cervera R, Filella X, Trejo O, de la Red G, et al. Th1/Th2 cytokine imbalance in patients with Sjogren syndrome secondary to hepatitis C virus infection. Semin Arthritis Rheum 2002;32(1):56–63.
[36] Marasini B, Gagetta M, Rossi V, Ferrari P. Rheumatic disorders and primary biliary cirrhosis: an appraisal of 170 Italian patients. Ann Rheum Dis 2001;60(11):1046–9.
[37] Benson GD, Kikuchi K, Miyakawa H, Tanaka A, Watnik MR, Gershwin ME. Serial analysis of antimitochondrial antibody in patients with primary biliary cirrhosis. Clin Dev Immunol 2004;11(2):129–33.
[38] Van Norstrand MD, Malinchoc M, Lindor KD, Therneau TM, Gershwin ME, Leung PS, et al. Quantitative measurement of autoantibodies to recombinant mitochondrial antigens in patients with primary biliary cirrhosis: relationship of levels of autoantibodies to disease progression. Hepatology 1997;25(1):6–11.