Anti-melanoma-associated Antigen A1 Autoantibodies Predict the Outcome of Patients With Idiopathic Inflammatory Myopathies Related Interstitial Lung Disease

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Research

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Abstract

Background

The discovery of novel autoantibody in patients with idiopathic inflammatory myopathies (IIMs) is of great significance for clinical subtype stratification and potential pathogenesis. Previous literatures have found that tumor-associated antigens were involved in the pathogenesis of IIM. Therefore, in this study, we aimed to explore the prevalence and clinical association of autoantibodies against melanoma-associated antigen A1 (MAGE-A1) in patients with IIM.

Methods

ELISA was performed to detect anti-MAGE-A1 autoantibodies in patients with IIM, systemic lupus erythematosus, rheumatoid arthritis, primary Sjögren syndrome, systemic sclerosis, and healthy controls, and the results were confirmed using the dot-immunoblotting assay. The association between anti-MAGE-A1 autoantibody and clinical characteristics was analyzed in IIM patients. T test, Mann-Whitney U test, double-sided Pearson's Chi-square, Fisher exact test, Spearman correlation analysis as well as the generalized estimating equation were applied in the statistical analyses.

Results

Anti-MAGE-A1 autoantibodies were detected in 5.03% (29/576) of all IIM patients, 4.60% (18/390) of dermatomyositis, 7.20% (5/69) of amyopathic dermatomyositis, and 5.10% (6/117) of polymyositis/immune-mediated necrotizing myopathy patients. The frequency of interstitial lung disease (ILD) was higher in anti-MAGE-A1-positive patients than those in anti-MAGE-A1-negative ones (82.8% vs 55%, p = 0.003). Anti-MAGE-A1-positive patients with IIM associated ILD had less dyspnea, more commonly asymptomatic ILD, and higher percentage of force vital capacity and diffusing lung capacity of carbon monoxcide of predicted values when compared to negative ones. The anti-aminoacyl-tRNA synthetase (ARS) coexisting anti-MAGE-A1-positive patients had common monocyclic disease course, while only anti-ARS-positive and anti-ARS coexisting anti-Ro52-positive patients had a more polycyclic disease course. None of anti-ARS-positive patients coexisting anti-MAGE-A1 antibody died during 10-year follow-up evaluation.

Conclusion

Anti-MAGE-A1 is a novel autoantibody associated with ILD in IIM patients. Anti-MAGE-A1-positive IIM patients with ILD appear to have good prognosis. Detection of anti-MAGE-A1 autoantibody may be useful in predicting the outcome of IIM patients with ILD.

Background

Idiopathic inflammatory myopathies (IIMs) are a group of heterogeneous systemic autoimmune diseases characterized by inflammatory cell infiltration in skeletal muscles, potentially resulting in multiple organ damage and a high degree of morbidity and mortality. Dermatomyositis (DM), polymyositis (PM), and immune-mediated necrotizing myopathies (IMNM) with distinct clinical, serological, and histopathological features are common subtypes in adult IIM patients.

Interstitial lung disease (ILD) is a common feature of IIM patients and a critical factor associated with disease severity and mortality. Myositis specific autoantibodies (MSAs) have been recognized as one of the most important biomarkers for clinical subtype stratification and potential factors involved in IIM pathogenesis(1–3). Some MSAs such as anti-aminoacyl-tRNA synthetase (ARS) and anti-melanoma differentiation-associated gene-5 (MDA5) autoantibodies are strongly associated with ILD and poor prognosis of IIM patients. Measurement of serum MSA levels could serve as a useful tool to predict the outcome of IIM. However, IIM patients with one MSAs can present different clinical process and prognosis. For example, patients with anti-MDA5 autoantibodies are at the highest risk of rapidly progressive ILD (RP-ILD) and have the worst prognosis among all IIM patients (4–7). However, anti-MDA5-positive patients with poor prognoses have significant increased serum ferritin level than those with favorable outcome (8–10). Moreover, previous study reported that IIM patients carrying anti-threonyl tRNA-synthetase (PL-7) and anti-Ro-52 autoantibodies have worse outcomes than those with anti-PL-7 autoantibody (11). These findings suggest the need to identify clinical biomarkers for IIM.

Melanoma-associated antigen-A1 (MAGE-A1), first reported as one of the cancer-testis antigen (CTAs) by Van Der Bruggen et al. in 1991(12), is a useful biomarker for tumor imaging and immunotherapy (13–15). *MEGE-A1* belongs to the *MAGE* family located on the X chromosome, which can be classified as type I and type II *MAGEs* (18). Under normal circumstances, *MEGE-A1* is expressed only in normal testes. However, the *MEGE-A1* promoter in humans is highly methylated. In cases of unstable demethylation, *MEGE-A1* is upregulated in various tumor tissues and is associated with tumor progression and a poor prognosis (15, 17, 18).

A subsequent study investigated the role of *MAGE* genes in autoimmune diseases. McCurdy et al. reported that MAGE-A1 is overexpressed in synovial fluid cells and peripheral blood mononuclear cells of juvenile rheumatic arthritis (JRA) both at the mRNA and the protein levels, suggesting that MAGE-A1 dysregulation is potentially associated with autoimmune disease and contributes to the pathogenesis of autoimmune diseases (19). Tumor-associated antigens, including transcription intermediary factor 1-γ (TIF1-γ), are potentially overexpressed in regenerating muscle fibers of IIM, and anti-TIF1-γ autoantibodies can be detected in the serum of IIM patients (20, 21). These results suggest that some tumor antigens may be converted to muscle antigens under unknown factors to stimulate the immune response for autoantibody production, thus mediating muscle fiber and organ damage associated with the clinical disease phenotype (22–24). However, no studies to date have investigated the role of MAGE-A1 in IIM.

This study aimed to investigate the association between anti-MAGE-A1 autoantibodies and the clinical features of IIM.

Methods

1. Patients

In total, 576 adults IIM patients, including 390 dermatomyositis (DM) patients, 69 amyopathic dermatomyositis (ADM) patients, and 117 polymyositis (PM) or immune-mediated necrotizing myopathy (IMNM) patients, admitted to the department of rheumatology at China-Japan Friendship hospital from 2008 July to 2018 March were enrolled in this study. All the patients fulfilled the 2017 EULAR/ACR IIM classification criteria (25). Since juvenile dermatomyositis and sporadic inclusion body myositis were rare in our cohort, these two IIM subgroups were excluded to reduce selection bias. The clinical data were retrospectively obtained from hospital medical records. Interstitial lung disease (ILD) was diagnosed in accordance with the features of high-resolution chest computed tomography (HRCT). RP-ILD was defined as the radiologic aggravation accompanied by progressive dyspnea and/or hypoxemia within 3 months of onset of respiratory symptoms (8, 26). Furthermore, 165 healthy controls (HCs) and 141 patients with other connective tissue diseases (CTD), including 40 systemic lupus erythematosus (SLE), 40 rheumatoid arthritis (RA), 40 primary Sjögren syndrome (pSS), and 21 systemic sclerosis (SSc) patients, were enrolled. Sera of all the aforementioned patients were obtained during every visit and stored at -80 ℃.

This study was approved by the Research Review Committee and the Ethical Review Committees of the China-Japan Friendship Hospital under the registration number 2016 – 117. Furthermore, written informed consent was obtained from all patients participating in this study.

2. Generation Of The Mage-a1 Peptides

Based on the gene name, corresponding human gene sequences were obtained from NCBI and their protein sequences were downloaded (msleqrslhc kpeealeaqq ealglvcvqa atssssplvl gtleevptag stdppqspqg asafpttinf trqrqpsegs ssreeegpst scileslfra vitkkvadlv gflllkyrar epvtkaemle sviknykhcf peifgkases lqlvfgidvk eadptghsyv lvtclglsyd gllgdnqimp ktgfliivlv miamegghap eeeiweelsv mevydgrehs aygeprkllt qdlvqekyle yrqvpdsdpa ryeflwgpra laetsyvkvl eyvikvsarv rfffpslrea lreeeegv). Thereafter, using the online prediction website IEBD Analysis Resource (http://tools.immuneepitope.org/main/), B cell epitopes were predicted and peptides with a segment length of 25aa (molecular weight, 2838 g/mol) (fpttinf trqrqpsegs ssreeegp) with the highest score were selected on the basis of the amino acid sequence and Hidden Markov Model. The selected peptide segments were then synthesized at Genscript Biotechnology (https://www.genscript.com.cn/), with a purity of ≥ 85%. Finally, the peptides were dissolved in MES buffer and stored at -30℃.

3. Establishment Of Anti-mage-a1 Elisa System

ELISA was performed herein. Synthesized MAGE-A1 polypeptides were incubated in vitro at 200 ng together with 100 µg 1-ethyl-3-(3-dimethylaminopropyl) per well in MES buffer (PH 6.0) at 4 °C overnight. After three washes with double steamed water, ELISA plates were blocked at 37 °C for 2 h with 5% skim milk powder. Next, 3 µl of patient sera was diluted with 2.5% milk (dilution 1:50) and added 100 µl of mixed solution to each well and incubated for 1 hour, followed by probing with goat anti-human IgG (dilution 1:20,000) (Abcam, Cambridge, UK) to detect autoantibodies. Serum and goat anti-human IgG were washed five times with 0.05% PBST. Finally, the absorbance was measured at 450 nm in a microplate reader. All the serum samples were analyzed more than twice to ensure consistency. Based on serial concentration of serum samples with a high titer of anti-MAGE-A1 antibodies, a standard curve was constructed, 1: 25 dilution was defined as 64U, 1: 50, 32U; similar to the antibody levels, as U/ml (see Supplementary Fig. 1, Additional File 1). If a particular sample presented as an outlier in the standard curve, further dilution was required. The cutoff level was set at 3.3737 U, which was determined through three standard deviations (SDs) above the average of 165 sera of HCs.

4. Validation Of Anti-mage-a1 Autoantibody

Commercial full-length recombinant MEAG-A1 protein (Origene, Rockville, USA) was subjected to a dot-immunoblotting assay to validate the results of ELISA among anti-MEGE-A1-positive patients and HCs. Recombinant MAGE-A1 protein diluted with PBS (dilution 1:50) was doted on a nitrocellulose membrane (200 ng/dot) at room temperature (24–28℃) for 5 min. Then the membrane was placed in 5% skim milk at room temperature for 2 hours. Afterwards, incubated 2 µl serum sample (dilution 1:500) at 4℃ overnight and washed the membrane for 6 times with 0.025%PBST solution. Thereafter, a secondary antibody against human sera (dilution 1:40000) was added. Finally, after repeating the washing step, ECL was used to visualize reactive dots.

5. Detection Of Msa/maas

A commercial immunoblot assay (Euroimmun, Luebeck, Germany) was used to detect MSAs, such as anti-TIF1γ, anti-nuclear matrix protein-2 (NXP-2), anti-small ubiquitin-like modifier-1 activating enzyme (SAE), anti-MDA5, anti-nucleosome remodeling deacetylase complex (Mi-2), anti-signal recognition particle (SRP), and anti-ARS, including anti-□histidyl-tRNA synthetase (Jo-1), anti-PL-7, anti-□alanyl tRNA-synthetase (PL-12), anti□-glycyl-tRNA synthetase (EJ), and □anti-isoleucyl-tRNA synthetase (OJ). Autoantibodies against 3-hydroxy-3-methylglutaryl-CoA reductase (HMGCR) were detected using a commercial ELISA kit (Inova Diagnostics, San Diego, CA, USA) in accordance with the manufacture's protocol. Other myositis-associated autoantibodies (MAAs), such as anti-Ro52, anti-Sjögren syndrome A (SSA) and -polymyositis-sclerosis (PM-Scl) were detected using commercial kits from Euroimmun (Luebeck, Germany) in accordance with the manufacturer's instructions.

6. Assessment Of Disease Activity

A cross-sectional study was performed to analyze the prevalence of anti-MAGE-A1 autoantibody and analyze the association between autoantibodies and clinical characteristics, while the longitudinal study primarily investigated the correlation between anti-MAGE-A1 autoantibody levels and disease activities.

A continuous 10 cm visual analog scale (VAS) for physician global assessment (PGA) of patients harboring the anti-MAGE-A1 autoantibody was used to evaluate disease activity in accordance with myositis core set measures (CSM) established by the International Myositis Assessment and Clinical Studies (IMACS) (27). Disease activity was assessed upon IIM diagnosis and during every follow-up visit. Disease courses were divided into the following four types: (1) monocyclic course, defined as patients retaining no clinical and biochemical signs of disease activity during 2-year follow-up after initial therapy; (2) polycyclic course, defined as patients presenting more than at least one relapses during 2-year follow-up; (3) chronic course, defined as active diseases on a 2-years visit after IIM diagnosis and no sign of disease remission despite regular treatment; (4) undefined, indicating that patient were followed-up for l < 2 years after diagnosis (28–30).

7. Statistical Analysis

Continuous variables were presented as mean ± standard deviation (SD) or median (interquartile range, IQR), and the Mann-Whitney U test was performed to compare non-normal distributed data. While categorical variables were described as numbers or percentages, they were compared using double-sided Pearson's Chi-square or Fisher exact test. Spearman correlation analysis was performed for correlation analysis in the cross-sectional study and the generalized estimating equation (GEE) was applied in the longitudinal study. P-values less than 0.05 were considered statistically significant. Data were visualized and analyzed using SPSS (version 25.0) and GraphPad Prism (version 8.0).

Results

1. Prevalence of anti-MAGE-A1 autoantibodies in IIM and other connective tissue diseases

Among 576 IIM and 141 patients with other CTD, the prevalence of anti-MAGE-A1 autoantibodies were 5.03% (29/576) in IIM, 15.0% (6/40) in SLE, 12.5% (5/40) in RA, 27.5% (11/40) in pSS and 38.1% (8/21) in SSc, respectively. The prevalence of the anti-MAGE-A1 autoantibody was significantly higher in IIM and other CTDs than in HCs ($p < 0.05$) (Fig. 1a). Further subgroup analysis revealed that the prevalence of the anti-MAGE-A1 autoantibody in DM, ADM, and PM (IMNM) was 4.6% (18/390), 7.2% (5/69), and 5.1% (6/117), respectively. No statistically significant differences in anti-MAGE-A1autoantibody levels were noted among three IIM subgroups ($p = 0.679$) (Fig. 1b). The dot-immunoblot assay for MAGE-A1 protein revealed reactive serum samples obtained from anti-MAGE-A1-positive patients, positive control and 1 HC (Fig. 1c).

2. Clinical Characteristics Of Anti-mage-a1-positive Iim Patients

Among IIM patients, the onset age of anti-MAGE-A1 positive patients was greater than that of anti-MAGE-A1 negative patients (median: 55.0 vs. 47.0, p = 0.006). No significant difference in clinical manifestations, including muscle weakness, skin rash, arthritis, dysphagia, and concomitant malignancy, were observed between anti-MAGE-A1-positive and -negative patients. Interestingly, ILD was more frequent among anti-MAGE-A1 positive patients than among -negative patients (82.8% vs. 55.0%, p = 0.003). Anti-MAGE-A1-positive patients also had MSAs and MAAs. However, no significant differences in the frequency of MSAs and MAAs and the levels of creatine kinase (CK), C-reactive proteins (CRP), and ferritin were observed between anti-MAGE-A1-positive and -negative patients (Table 1).

Table 1
Clinical characteristics of patients with idiopathic inflammatory myopathies with and without anti-MAGE-A1 autoantibody

Variables	Anti-MAGE-A1-positive (N = 29)	Anti-MAGE-A1-negative (N = 547)	P value
Clinical subsets			
DM	62.1%(18/29)	68%(372/547)	0.801
ADM	17.2%(5/29)	12%(64/547)	0.667
PM/IMNM	20.7%(6/29)	20%(111/547)	0.999
General features			
Female	76%(22/29)	68%(374/547)	0.396
Onset Age(years)	55.0(41.5–65)	47.0(36–57)	0.006
Course of disease(months)	10(2.5–25.5)	8(3–24)	0.908
Clinical manifestations			
Muscle weakness	69.0%(20/29)	65% (353/547)	0.626
Myalgia	24.1%(7/29)	37% (203/547)	0.157
Heliotrope rash	24.1%(7/29)	41.1%(225/547)	0.069
Gottron's papule	44.8%(13/29)	43.3% (237/547)	0.874
V-neck sign	41.4%(12/29)	36.6% (200/547)	0.600
Shawl sign	37.9%(11/29)	26.8% (147/547)	0.193
Mechanic hands	27.6%(8/29)	23.9% (131/547)	0.656
Skin ulcer	13.8%(4/29)	10.4% (57/547)	0.791
Raynaud phenomenon	10.3%(3/29)	5.9% (32/547)	0.556
Fever	20.7%(6/29)	24.1%(132/547)	0.672
Dysphagia	37.9%(11/29)	23.2% (127/547)	0.07
Arthritis	27.6%(8/29)	21.0% (115/547)	0.401
Malignancy	3.4%(1/29)	9.9% (54/547)	0.411
ILD	82.8%(24/29)	55.0% (301/547))	0.003
Laboratory parameters			
CK levels (IU/L))[a]	119(58-1313.5)	131.5(46-1122.5)	0.903

Variables	Anti-MAGE-A1-positive (N = 29)	Anti-MAGE-A1-negative (N = 547)	P value
CRP levels(mg/dl)[b]	0.3615(0.1768–1.1975)	0.433(0.206–0.994)	0.639
Ferritin(ng/ml)[c]	226.9(109.9-560.8)	187.1(80.55–528.6)	0.730
ANA	51.7%(15/29)	28.9%(158/547)	0.009
MSAs			
Anti-ARS	27.6%(8/29)	17.2%(92/534)	0.152
Anti-Jo1	62.5%(5/8)	47.8%(44/92)	0.669
Anti-PL-7	12.5%(1/8)	27.2%(25/92)	0.626
Anti-PL-12	12.5%(1/8)	7.6%(7/92)	0.500
Anti-EJ	12.5%(1/8)	17.4%(16/92)	1.000
Anti-OJ	0(0/8)	0(0/92)	/
Anti-TIF1γ	10.3%(3/29)	15.4%(82/534)	0.645
Anti-MDA5	13.8%(4/29)	15.2%(81/534)	1.000
Anti-Mi-2	3.4%(1/29)	5.6%(30/534)	0.939
Anti-NXP2	6.9%(2/29)	7.5%(40/534)	1.000
Anti-SAE	3.4%(1/29)	2.1%(11/534)	0.472
Anti-HMGCR	3.4%(1/29)	3.6%(19/534)	1.000
Anti-SRP	6.9%(2/29)	7.3%(39/534)	1.000
MSA-negative	24.1%(7/29)	26.6%(142/534)	0.779
MAAs			
Anti-Ro52	31.0%(9/29)	22.1%(120/542)	0.264
Anti- SSA	13.8%(4/29)	10.3%(56/544)	0.773
Anti-PM-Scl	3.4%(1/29)	1.1%(6/544)	0.306

MAGE-A1, Melanoma-associated antigenA1; DM, dermatomyositis; ADM, amyopathic dermatomyositis; PM, polymyositis; IMNM, immune-mediated necrotizing myopathy; ILD, interstitial lung disease; CK, creatine kinase; CRP, C-reactive protein; ANA, anti-nuclear antibody; MSA, myositis specific autoantibodies; ARS, aminoacyl-tRNA synthetases; Jo1, histidyl-tRNA synthetase; PL-7, threonyl-tRNA synthetase; PL-12, alanyl-tRNA synthetase; EJ, glycyl-tRNA synthetase; OJ, isoleucyl-tRNA synthetase; TIF1γ, transcription intermediary factor 1γ; MDA5, melanoma differentiation-associated gene-5; Mi-2, nucleosome remodeling deacetylase complex; NXP-2, nuclear matrix protein-2; SAE, small ubiquitin-like modifier-1 activating enzyme; HMGCR, 3-hydroxy-3-methylglutaryl-CoA reductase; SRP, signal recognition particle; MAA, myositis associated autoantibodies; SSA, Sjögren syndrome A; PM-Scl, polymyositis-sclerosis.

Variables	Anti-MAGE-A1-positive (N = 29)	Anti-MAGE-A1-negative (N = 547)	P value
[a]Data was available for 569 patients			
[b]Data was available for 554 patients			
[c]Data was available for 372 patients			

3. Comparison of clinical features of IIM-associated ILD patients with and without anti-MAGE-A1 autoantibodies

In total, 325 IIM patients had ILD in our cohort. On comparing the clinical characteristics of patients with IIM-associated ILD (IIM-ILD) with and without anti-MAGE-A1 autoantibodies, no significant differences in clinical manifestations, such as muscle weakness, skin rash, arthritis, and dysphagia or laboratory findings including serum CK levels or MSAs and MAAs, were observed between the two groups (See Supplementary Table 1, Additional File 1). Interestingly, anti-MAGE-A1-positive patients had less dyspnea and more common asymptomatic ILD at the diagnosis of IIM than anti-MAGE-A1-negative patients (20.8% vs 47.2%, 79.2% vs 52.2%, p = 0.013 and 0.011, respectively). The patients were diagnosed with ILD based on the positive findings of HRCT. Furthermore, the PFT revealed that anti-MAGE-A1-positive patients tended to have a higher mean percentage of force vital capacity (FVC%) and carbon monoxide diffusing capacity (DLco%) of predicted values than anti-MAGE-A1-negative patients (median: 90.3 vs 78.1, 74 vs 59.4, p = 0.188 and 0.034, respectively) (Table 2).

Table 2
ILD Characteristics in IIM-ILD patients, with and without anti-MAGE-A1 autoantibody

Variables	Anti-MAGE-A1-positive (N = 24)	Anti-MAGE-A1-negative (N = 301)	P values
ILD presenting			
Asymptomatic ILD	79.2%(19/24)	52.2%(157/301)	0.011
Dyspnea	20.8%(5/24)	47.2%(142/301)	0.013
Dry cough	20.8%(5/24)	34.2%(103/301)	0.180
RP-ILD	8.3% (2/12)	15.3% (46/31)	0.532
PFTs	N = 16	N = 177	
FVC% of predicted value	90.3 (73.2-104.7)	78.1 (64.9–100.0)	0.188
DLco% of predicted value	74.0 (52.9–78.2)	59.4 (46.2–70.4)	0.034

MAGE-A1, Melanoma-associated antigenA1; IIM, idiopathic inflammatory myopathies; ILD, interstitial lung disease; IIM-ILD, IIM associated ILD; RP-ILD, rapidly progressive ILD; PFTs, pulmonary function tests; FVC, forced vital capacity; DLco, carbon monoxide diffusing capacity.

4. Role of anti-MAGE-A1 autoantibody for predicting the prognosis of IIM-associated ILD patients

As above results, anti-MAGE-A1-positive patients with ILD can coexist with other MSAs, which the most common MSA was anti-ARS antibody (See Supplementary Table 1, Additional File 1). In addition, previous studies demonstrated that anti-Ro52 also frequently coexisted with anti-ARS antibody and associated with poor outcome of anti-ARS-positive patients with ILD. Therefore, the characteristics of anti-ARS-positive IIM-ILD patients with and without anti-MAGE-A1 antibody, as well as anti-Ro52 antibody were analyzed. The predicted values of FVC% and DLco% were higher in cases of ARS with anti-MAGE-A1 antibody than in those with only ARS (95.2 ± 13.8 vs 73.7 ± 19.6 and 71.3 ± 12.6 vs 52.7 ± 16.9, p = 0.035 and 0.027, respectively), while no differences were observed between ARS-positive patients with or without the anti-Ro52 autoantibody (Table 3). Furthermore, in the longitudinal study, the follow-up duration was 57(39–78) months. Patients both with anti-ARS and anti-MAGE-A1 antibodies presented a more monocyclic disease course compared those with only anti-ARS (50% vs 10.5%, p = 0.012). Meanwhile, anti-ARS-positive patients with anti-Ro52 antibody presented a more polycyclic disease course compared to those with anti-MAGE-A1 antibody (62.2% vs 12.5%, p = 0.017) (Table 3).

Table 3
Comparisons of anti-ARS-positive IIM-ILD patients, with and without anti-MAGE-A1 and anti-Ro52 autoantibodies

	Anti-ARS-positive IIM-ILD patients (N = 94)					
Variables	**Anti-MAGE-A1 positive (N = 8)**	**Anti-MAGE-A1 negative (N = 86)**	**P value**	**Anti-Ro52 positive (N = 37)**	**Anti-Ro52 negative (N = 57)**	**P value**
ILD presenting						
Asymptomatic	75%(6/8)	16.3% (14/86)	0.001	21.6% (8/37)	21.1% (12/57)	0.947
Dyspnea	25%(2/8)	65.1% (56/86)	0.064	67.6% (25/37)	57.9% (33/57)	0.346
Cough	25%(2/8)	62.8% (54/86)	0.088	54.1% (20/37)	63.2% (36/57)	0.380
PFTs	N = 4	N = 64/63		N = 29	N = 39/38	
FVC% of predicted value	95.2 ± 13.8	73.7 ± 19.6	0.035	74.8 ± 20.5	75.1 ± 19.7	0.949
DL_{CO}% of predicted value	71.3 ± 12.6	52.7 ± 16.9	0.027	51.9 ± 18.8	55.3 ± 16.0	0.432
Disease course						
Monocyclic course	50%(4/8)	10.5% (9/86)	0.012	16.2% (6/37)	10.5% (6/57)	0.623
Polycyclic course	12.5%(1/8)	55.8% (48/86)	0.026	62.2% (23/37)*	59.6% (34/57)	0.832
Chronic continuous course	25%(2/8)	11.6% (10/86)	0.596	8.1%(3/37)	15.8% (9/57)	0.439
Undefined	12.5%(1/8)	22.1% (19/86)	1.000	13.5% (5/37)	14.0% (8/57)	1.000

MAGE-A1, Melanoma-associated antigenA1; ARS, aminoacyl-tRNA synthetases; IIM, idiopathic inflammatory myositis; ILD, interstitial lung disease; IIM-ILD: IIM associated ILD; PFTs, pulmonary function tests; FVC, forced vital capacity; DLco, carbon monoxide diffusing capacity.

*$p < 0.05$ compared to anti-ARS-positive patients with anti-MAGE-A1 antibody.

Moreover, none of the eight patients with anti-ARS and anti-MAGE-A1 antibodies died during long-term follow-up. The overall survival rates of IIM-ILD patients both with anti-ARS and anti-MAGE-A1, anti-ARS

and anti-Ro52 and only with anti-ARS autoantibodies were 100%, 79% and 74.3%, respectively, on 10-year follow-up (Fig. 2).

The second most common MSA that coexisting with anti-MAGE-A1 autoantibody was anti-MDA5 antibody (See Supplementary Table 1, Additional File 1), however, no significant differences in the characteristics of ILD and disease course were observed between anti-MDA5-positive ILD patients with or without anti-MAGE-A1 autoantibody (See Supplementary Table 2, Additional File 1).

5. Correlation between serum anti-MAGE-A1 autoantibody levels and disease activities

In the cross-sectional study, no correlation was found between serum anti-MAGE-A1 level and PGA-VAS ($r=-0.049$, $p = 0.800$) among anti-MAGE-A1-positive patients. However, sera from 11 patients followed up at least more than twice were obtained to monitor changes in anti-MAGE-A1 autoantibody levels. Among these patients, four out of 11 were anti-MDA5-positive, two were anti-ARS-positive, two were MSA-negative, and one was anti-NXP2-, anti-HMGCR-, and anti-SRP-positive. The levels of anti-MAGE-A1 autoantibodies decreased with a reduction in disease activity. Anti-MAGE-A1 autoantibody levels were positively correlated with PGA-VAS on multiple GEE analysis performed in the longitudinal study ($\beta = 1.071$, $p < 0.0001$) (Fig. 3).

Discussion

The present study shows that that the incidence of anti-MAGE-A1 autoantibody is approximately 5% in IIM patients, and this autoantibody can be present in patients with all adult subtypes of IIM including DM, ADM, and PM (IMNM). Furthermore, our results show that anti-MAGE-A1 antibody are found in patients with other CTDs such as SLE, RA, SS, and SSc, and these can coexist with MSAs, indicating that anti-MAGE-A1 autoantibody are MAAs. In addition, this study shows that the prevalence of ILD is higher among anti-MAGE-A1-positive IIM patients (82.8%) than among anti-MAGE-A1-negative patients. Numerous autoantibodies, including MSAs such as anti-ARS and anti-MDA5 antibodies, as well as anti-Ro52 and anti-Ku among MAAs, are strongly associated with IIM-ILD (11, 31–35). On the other hand, IIM-ILD patients with different serological MSAs and MAAs present various clinical features and prognoses, indicating the heterogeneity of IIM-ILD. This study shows that anti-MAGE-A1 autoantibody may serve as a potential serological biomarker for IIM-ILD. Anti-MAGE-A1-positive patients had more asymptomatic respiratory manifestations and higher FVC% and/or DLco% of predicted values on the PFT than anti-MAGE-A1-negative patients, suggesting potential mild lung involvement in that IIM patients with anti-MAGE-A1 autoantibody.

Anti-MAGE-A1 is an MAA rather than an MSA and can coexist with MSA, especially with ARS herein. Recent studies reported that the anti-ARS autoantibody is a serological biomarker of anti-synthetase syndrome (ASS) characterized by ILD, fever, Raynoud's phenomenon, arthralgia, mechanic hands, myositis, and ARS, generally being refractory and resulting in relapse (36, 37). Our longitudinal study shows that anti-ARS/anti-MAGE-A1-double-positive patients presented a more monocyclic disease course than the anti-ARS-positive patients on 2-year follow-up evaluation. Moreover, the survival rate of patients

both with anti-ARS and anti-MAGE-A1 autoantibodies was 100% on 10-year long-term follow-up, while the overall survival rate of anti-ARS-positive patients without anti-MAGE-A1 antibody was 75%. However, differences in clinical features between IIM-ILD patients with and those without anit-MAGE-A1 antibody were not observed among those with anti-MDA5 autoantibody. These results suggest that anti-MAGE-A1 antibody may predict a favorable outcome for IIM patients especially for ASS patients with ILD.

Furthermore, this study shows that serum anti-MAGE-A1 autoantibody levels were positively correlated with disease activity, suggesting that anti-MAGE-A1 autoantibodies may contribute to the pathogenesis of IIM, and monitoring of anti-MAGE-A1 levels may help guide corresponding clinical decisions. MAGE-A1 is a cancer-testis antigen with unclear physiological function. As previously reported studies, the pathogenesis of fibrotic lung diseases formation often involves aberrant regulation of cytokines including transforming growth factor-β (TGF-β), a key cytokine promoting fibrosis. Furthermore, ubiquitination plays an important role in the pathogenesis of fibrotic diseases in a TGF-β-dependent manner (38). MAGE-A1 can assemble with the E3 RING of ubiquitin ligase to form MAGE-RING ligase to promote ubiquitination (16, 39). Interestingly, previous studies have reported that Ro52 antigen was an E3 ubiquitin ligase (40, 41), which is recognized by another IIM-ILD-associated MAA-anti-Ro52 autoantibody. MAGE-A1 and Ro52 may play similar roles in the pathogenesis of ILD through ubiquitination. However, the mechanism underlying the role of the autoantibodies in the pathogenesis of IIM warrants further investigation.

This study has some limitations. First, ELISA was performed to detect the anti-MAGE-A1 autoantibody in IIM, which may have resulted in unspecific binding of serum to MAGE-A1 peptide, yielding false-positive results. Therefore, the dot-immunoblot assay was performed to validate the results of ELISA. Because of the low molecular weight of MAGE-A1 peptide, the full-length recombinant MAGE-A1 protein was used for the dot-immunoblot assay herein. Although the results of ELISA and dot-immunoblot assay are not highly consistent, the positive reaction of dot-immunoblot indicated that anti-MAGE-A1-positive sera truly bind to MAGE-A1 protein. Future studies are required to use approaches that couple MAGE-A1 peptide to a protein for the immunoprecipitation assay to confirm the results of ELISA. Second, the prevalence of anti-MAGE-A1 autoantibody in IIM was not as high as expected. The association between the anti-MAGE-A1 autoantibody and clinical characteristics, as well as that between the anti-MAGE-A1 autoantibody and other MSA and/or MAAs in IIM warrant further investigation in future studies with larger patient cohorts. Moreover, it is necessary to conduct a long-term follow-up study to evaluate the effect of the anti-MAGE-A1 autoantibody on the prognosis of IIM-associated ILD, especially among non-anti-ARS-positive patients.

Conclusion

In conclusion, anti-MAGE-A1 autoantibody can exist in 5.03% of IIM patients and are associated with ILD. This study shows that anti-MAGE-A1-positive patients with IIM have milder pulmonary involvement and present favorable outcomes, especially those with anti-ARS antibody. Future studies are required to

comprehensively investigate the clinical significance of anti-MAGE-A1 autoantibodies and their precise role in the pathogenesis of IIM in multicenter cohorts.

Abbreviations

MAGE-A1
Melanoma-associated antigen-A1; IIM:idiopathic inflammatory myopathies; DM:dermatomyositis; PM:polymyositis; ADM:amyopathic dermatomyositis; IMNM:immune-mediated necrotizing myopathy; ILD:interstitial lung disease; RP-ILD:rapidly progressive ILD; IIM-ILD:IIM associated ILD; MSA:myositis specific autoantibody; ARS:aminoacyl-tRNA synthetase; Jo-1:histidyl-tRNA synthetase; PL-7:threonyl tRNA-synthetase; PL-12:alanyl tRNA-synthetase; EJ:glycyl-tRNA synthetase; OJ:isoleucyl-tRNA synthetase; MDA5:melanoma differentiation-associated gene-5; TIF1-γ:transcription intermediary factor 1-γ; NXP-2:nuclear matrix protein-2; Mi-2:nucleosome remodeling deacetylase complex; SAE:small ubiquitin-like modifier-1 activating enzyme; SRP:signal recognition particle; HMGCR:3-hydroxy-3-methylglutaryl-CoA reductase; MAA:myositis associated autoantibody; SSA:Sjögren syndrome A; PM-Scl:polymyositis-sclerosis; CTA:cancer-testis antigen; HRCT:high-resolution chest computed tomography; HC:healthy control; CTD:connective tissue disease; SLE:systemic lupus erythematosus; RA:rheumatoid arthritis; JRA:juvenile rheumatic arthritis; pSS:primary Sjögren syndrome; SSc:systemic sclerosis; VAS:visual analog scale; PGA:physician global assessment; CSM:core set measures; IMACS:International Myositis Assessment and Clinical Studies; SD:standard deviation; IQR:interquartile range; GEE:generalized estimating equation; CK:creatine kinase; CRP:C-reactive proteins; IIM-ILD:IIM-associated ILD; FVC:force vital capacity; DLco:carbon monoxide diffusing capacity; ASS:anti-synthetase syndrome; TGF-β:transforming growth factor-β.

Declarations

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Authors' contributions

YS designed the study and was a major contributor in writing the manuscript, WJ, HY participated in data collection and data analysis, HC, QP, YW and HY participated in the design of this study and experimental materials as well as technologies, GW and XL participated in experimental design and data analysis. All

authors have contributed to the last version of the manuscript. The authors read and approved the final manuscript.

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Availability of data and materials

All data generated or analysed during this study are included in this published article [and its supplementary information files].

Ethics approval and consent to participate

This study was approved by the Research Review Committee and the Ethical Review Committees of the China-Japan Friendship Hospital under the registration number 2016-117. Furthermore, written informed consent was obtained from all patients participating in this study.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Figures

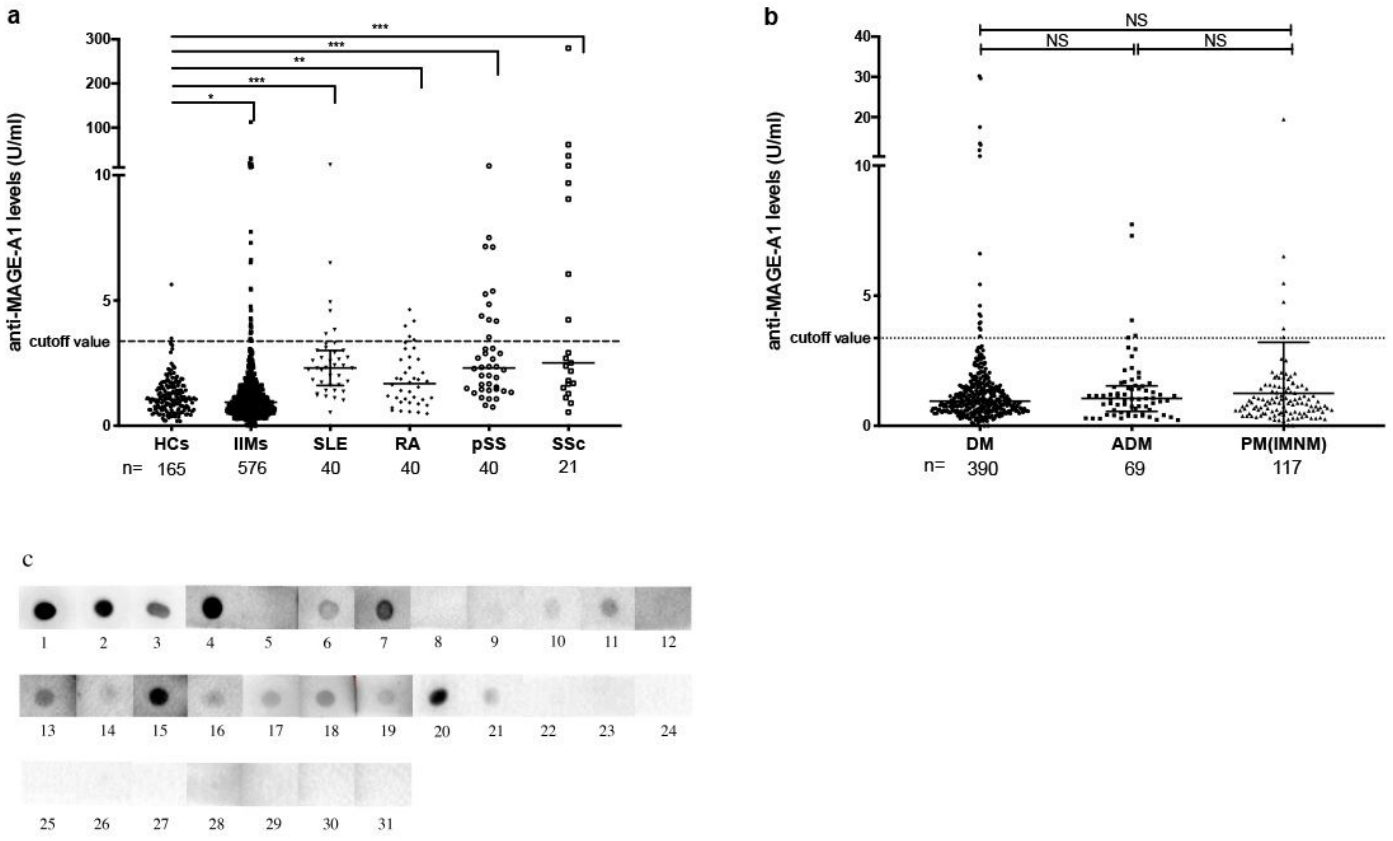

Figure 1

The prevalence of anti-MAGE-A1 autoantibodies in IIM and CTD patients and healthy control. a) The titers of anti-MAGE-A1 autoantibodies of sera from IIM(n=576), SLE(n=40), RA(n=40), pSS(n=40), SSc(n=21) and HCs(n=165). The horizontal dotted line set at 3.3737 represents the cutoff value which was defined as the mean value of sera anti-MAGE-A1 levels in HCs plus 3 times the SD. b) The prevalence of anti-MAGE-A1 autoantibodies in DM, ADM and PM(IMNM) respectively. c)Commercial full-length MAGE-A1 protein was used in dot-immunoblotting assay to confirmed the sera from IIMs patients and HCs by ELISA assay. Dot 1 performed as positive control, dot 2-30 performed serum from IIM patient with anti-MAGE-A1 antibodies detected by ELISA, dot 31 displayed serum from HC. MAGE-A1, melanoma-associated antigenA1; IIMs, idiopathic inflammatory myopathies; DM, dermatomyositis; ADM, amyopathic dermatomyositis; PM, polymyositis; IMNM, immune-mediated necrotizing myopathy; CTD, connective tissue disease; SLE, systemic lupus erythematosus; RA, rheumatoid arthritis; pSS, primary Sjögren syndrome; SSc, systemic sclerosis; HCs, healthy controls; ELISA, enzyme-linked immunosorbent assay; SD, standard deviation.

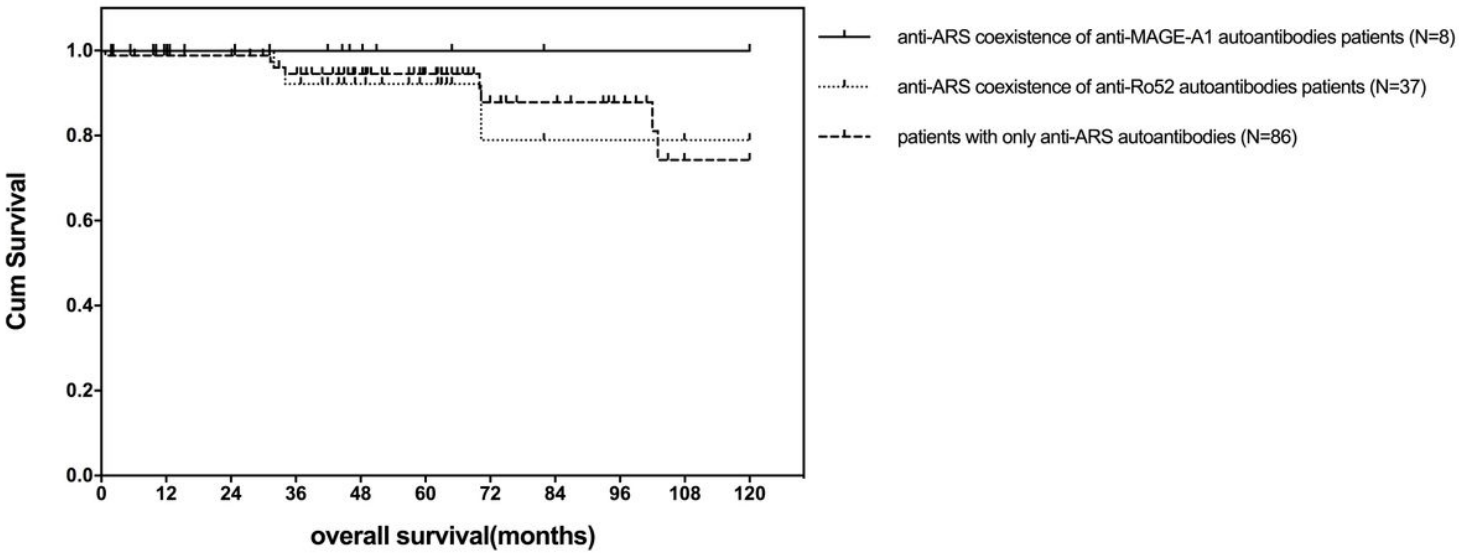

Figure 2

Kaplan-Meier curves of anti-ARS positive IIM associated ILD patients. IIM, idiopathic inflammatory myositis; ILD, interstitial lung disease; MAGE-A1, melanoma-associated antigenA1; ARS, aminoacyl-tRNA synthetases.

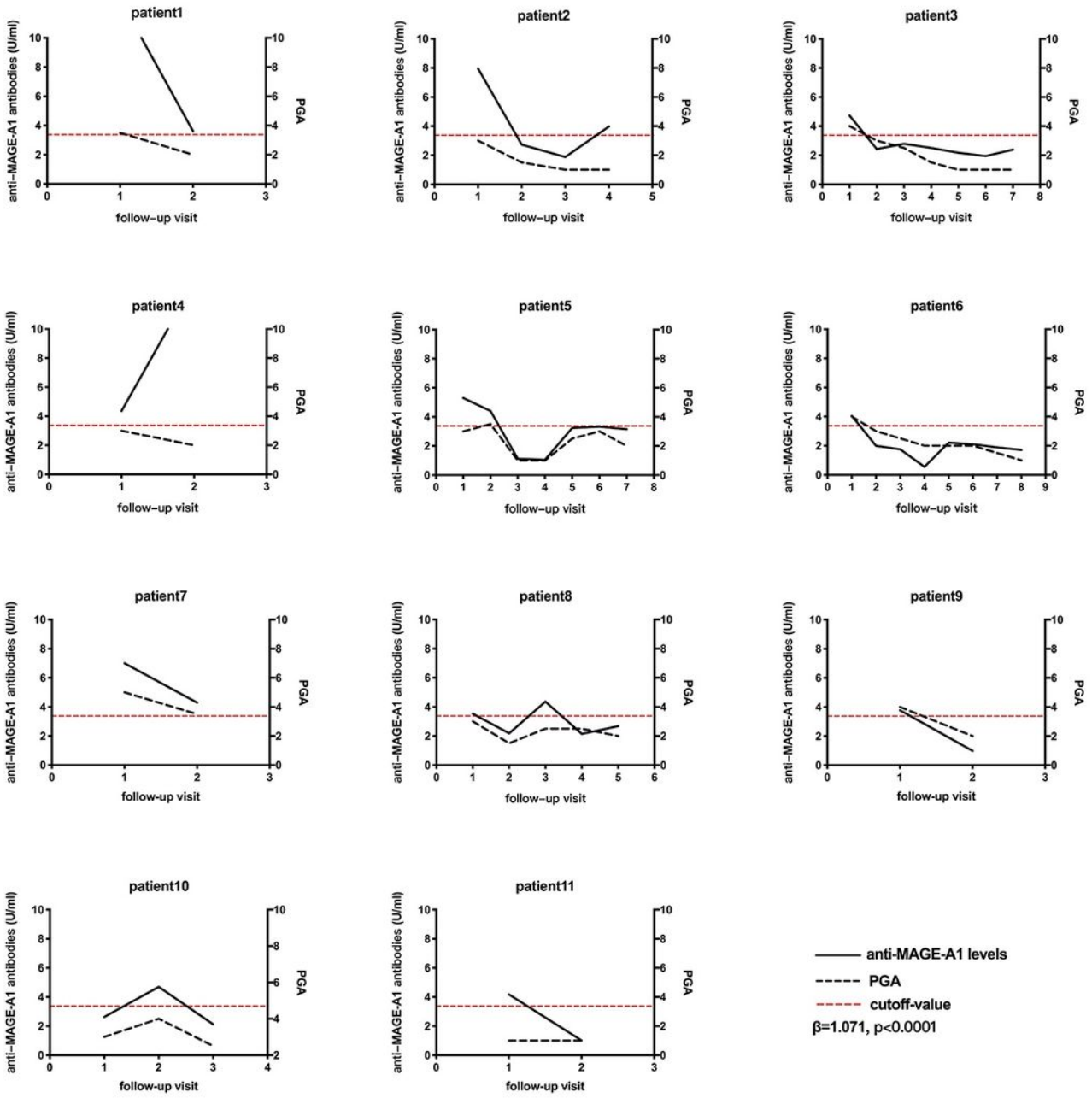

Figure 3

Longitudinal analysis of sera anti-MAGE-A1 levels and PGA VAS scores. MAGE-A1, melanoma-associated antigenA1; PGA, physician global assessment; VAS, visual analog scale.

Supplementary Files

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