Clinical features of thirty-two patients with anti-melanoma differentiation-associated gene 5 antibodies

W. Huang[1], F. Ren[1], Q. Wang[2], L. Luo[1], J. Zhou[1], D. Huang[1], Z. Pan[3], L. Tang[1]

[1]Department of Rheumatology and Immunology, The Second Affiliated Hospital of Chongqing Medical University, Chongqing, China; [2]Department of Rheumatology and Immunology, Peking Union Medical College Hospital, Beijing, China; [3]Department of Endocrinology and Nephrology, The Seven People's Hospital, Chongqing, China.

Abstract

Objective

To investigate the clinical characteristics of patients positive for anti-melanoma differentiation-associated gene 5 (MDA5) antibodies, and to analyse the potential pathogenesis of anti-MDA5 antibodies.

Methods

The clinical manifestations, serological tests, imaging features, treatments, and prognoses of 32 anti-MDA5 antibody-positive patients diagnosed in the Rheumatology and Immunology Department of the Second Affiliated Hospital of Chongqing Medical University from September 2015 to August 2018 were analysed.

Results

Of the 32 anti-MDA5 antibody-positive patients, eleven patients were clinically diagnosed with interstitial pneumonia with autoimmune features (IPAF), ten patients were diagnosed with clinically amyopathic dermatomyositis (CADM), six patients were diagnosed with dermatomyositis (DM) and five patients were diagnosed with anti-synthetase syndrome (ASS). Thirty patients had various degrees of pulmonary interstitial changes. The incidence of mortality, subcutaneous emphysema, hoarseness and dysphagia in patients who were positive for both anti-MDA5 and anti-Ro52 antibodies was significantly higher than in patients positive for only anti-MDA5 antibodies. The anti-MDA5 antibody-positive IPAF patients had a very poor prognosis, and mortality in these patients was as high as 54.55%.

Conclusion

Anti-MDA5 antibodies are closely related to interstitial lung disease (ILD). The presence of both anti-MDA5 and anti-Ro52 antibodies indicates poor prognosis.

Key words

anti-melanoma differentiation-associated gene 5 antibody, interstitial lung disease pathogenesis

*Wenhan Huang, MD**
*Feifeng Ren, MD**
Qian Wang, PhD
Lei Luo, MD
Jun Zhou, MD
Dongmei Huang, MD
Zhuma Pan, MD
Lin Tang, PhD

**These authors contributed equal to the paper.*

Please address correspondence to:
Dr Lin Tang,
Department of Rheumatology and Immunology,
The Second Affiliated Hospital of Chongqing Medical University,
76 Linjiang Road,
400010 Chongqing, China.
E-mail: hopetang@163.com

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Introduction

In 2005, Sato *et al*. (1) identified a 140 kDa peptide in the serum of Japanese patients with clinically amyopathic dermatomyositis (CADM) and named it the anti-CADM-140 antibody. In 2009, the Sato team found that the RNA helicase encoded by melanoma differentiation-associated gene 5 (MDA5) was the target antigen of the anti-CADM-140 antibodies (2). Therefore, the anti-CADM-140 antibody is currently known as anti-MDA5 antibody. Anti-MDA5 antibody is also found in patients with systemic lupus erythematosus (3), dermatomyositis (4) and other autoimmune diseases, and thus is gaining greater attention in the clinic.

Current studies (5, 6) show that the human body relies on pattern recognition receptors (PRRs) to distinguish pathogens from native tissue. These receptors are classified into two categories: membrane-bound receptors such as the Toll-like receptor family and cytoplasmic PRRs including RIG-I-like receptors, DNA sensors and NOD-like receptors. MDA5 is an important member of the RIG-I-like receptor family and plays a protective role by activating downstream signalling pathways and promoting anti-viral responses.

Since the discovery of anti-MDA5 antibodies, an increasing number of clinical studies have reported that these antibodies are closely associated with inflammatory myopathies (7) and the rapid progression of interstitial lung disease (8, 9). Patients positive for anti-MDA5 antibodies have a poor prognosis and high mortality. The role of the anti-MDA5 antibodies in the human body remains unclear, and it is not known if these antibodies block the protective effect of PRRs, leading to severe clinical symptoms. In this study, we summarised the clinical manifestations and outcomes of 32 anti-MDA5 antibody-positive patients and analysed the potential pathogenic mechanisms. This study may increase our understanding of anti-MDA5 antibodies and provide valuable scientific data for future studies.

Patients and methods

Clinical information

From September 2015 to August 2018, a total of 32 patients in the Second Affiliated Hospital of the Chongqing Medical University were found to be positive for anti-MDA5 antibodies. These 32 patients were all inpatients with complete clinical data and were included in this study. All patients were informed that their data would be used for possible studies or analyses, and all 32 patients consented to publish their data. Ethical approval was not required in accordance with the policy of our institution. In this study, the diagnosis of polymyositis/dermatomyositis (PM/DM), clinically amyopathic dermatomyositis (CADM) and anti-synthetase syndrome (ASS) were based on the idiopathic inflammatory myopathy (IIM) criteria (10), the diagnosis of interstitial pneumonia with autoimmune features (IPAF) was based on the 2015 consensus classification criteria of the European Society of Respiratory Diseases/American Thoracic Society (11). We used the OMRMUN assay kit to detect anti-MDA5 antibodies; a result of + was considered to be anti-MDA5 antibody-positive, while a result of - or ± was considered to be anti-MDA5 antibody-negative. High resolution computed tomography was used to perform chest radiography in all 32 patients.

Statistical analysis

All analyses were performed using SPSS 19.0 (IBM, Armonk, NY, USA). Patient characteristics, clinical symptoms and signs are presented as means ± SD or as percentages. Significance was assessed using Fisher's exact test, and values of $p<0.05$ were considered significant.

Results

Among the 32 anti-MDA5 antibody-positive patients, 12 were male (37.5%), 20 were female (62.5%), and the ratio of males to females was 1:1.67. The age range of disease onset was 19–73 years, with an average age of onset of 51.84±11.95 years (51.33±9.77 years for males and 52.15±13.32 years for females). The basic diseases of the 32 cases included eleven cases of IPAF (34.38%), ten cases of CADM (31.25%), six cases of DM (18.75%) and five cases of ASS (15.62%).

Table I. General information of 32 anti-MDA5 antibody-positive patients.

No	Sex	Age	MDA5	Ro-52	Antibodies	ILD	Diagnosis	Treatment	Prognosis
1	F	60	+	+		RP-ILD	IPAF	Steroid pulse*, PSL, CTX, IVIG	Dead
2	F	51	+		JO-1	Chronic	ASS	PSL, CTX, IVIG	Alive
3	F	19	+			Chronic	CADM	PSL	Alive
4	F	50	+	+	PL-12	-	ASS	PSL	Alive
5	F	48	+	+	EJ	Chronic	ASS	PSL, CTX	Alive
6	F	37	+			Chronic	CADM	PSL, TAC	Alive
7	F	50	+	+		RP-ILD	CADM	Steroid pulse, PSL, TAC, CTX, IVIG	Dead
8	F	63	+	+		RP-ILD	IPAF	Steroid pulse, PSL, CTX, IVIG	Dead
9	F	62	+	+		Chronic	IPAF	PSL, CTX	Alive
10	F	42	+	+		-	CADM	PSL, MTX	Alive
11	F	51	+	+		RP-ILD	CADM	Steroid pulse, PSL, CTX, IVIG	Dead
12	F	63	+	+		Chronic	IPAF	no treatment	Alive
13	F	63	+	+		RP-ILD	DM	Steroid pulse, PSL, CTX	Dead
14	F	48	+			Chronic	CADM	PSL, CTX	Alive
15	F	73	+			Chronic	IPAF	no treatment	Alive
16	F	67	+			Chronic	CADM	TGP	Alive
17	F	49	+			Chronic	IPAF	PSL, CTX	Alive
18	F	67	+		OJ	RP-ILD	ASS	PSL, CTX	Alive
19	F	50	+			RP-ILD	IPAF	PSL, CTX	Alive
20	F	30	+	+		RP-ILD	CADM	Steroid pulse, PSL, CTX, IVIG	Alive
21	M	46	+	+		RP-ILD	CADM	Steroid pulse, PSL, CsA, CTX	Alive
22	M	55	+	+		RP-ILD	IPAF	Steroid pulse, PSL, TAC, CTX	Dead
23	M	69	+	+		RP-ILD	DM	Steroid pulse, PSL, CTX, IVIG	Alive
24	M	54	+	+		RP-ILD	DM	Steroid pulse, PSL, CTX, IVIG	Alive
25	M	51	+	+		RP-ILD	DM	PSL, CTX	Alive
26	M	52	+	+		RP-ILD	IPAF	Steroid pulse, PSL, CTX, IVIG	Dead
27	M	50	+	+	JO-1	RP-ILD	ASS	PSL, CTX	Alive
28	M	59	+	+		RP-ILD	IPAF	Steroid pulse, PSL, CTX	Dead
29	M	46	+	+		RP-ILD	DM	Steroid pulse, PSL, CTX, IVIG	Alive
30	M	58	+	+		RP-ILD	CADM	Steroid pulse, PSL, CTX, MTX	Alive
31	M	48	+	+		RP-ILD	IPAF	Steroid pulse, PSL, TAC, CTX, IVIG	Dead
32	M	28	+			Chronic	DM	PSL, TAC	Alive

M: male; F: female; ILD: interstitial lung disease; RP-ILD: rapidly progressive ILD; IPAF: interstitial pneumonia with autoimmune features; DM: dermatomyositis; CADM: clinically amyopathic dermatomyositis; ASS: anti-synthetase syndrome; PSL: prednisolone; TAC: tacrolimus; CsA: cyclosporine; CTX: cyclophosphamide; TGP: Total glucosides of white paeony capsule; IVIG: intravenous immunoglobulin.
*Steroid pulse therapy: methylprednisolone 500mg daily for 3 days.

Among the 32 anti-MDA5 antibody-positive patients, 22 were also positive for anti-Ro52 antibodies (68.75%) and five cases were positive for anti-synthetase antibodies (15.63%). The clinical manifestations of the 32 anti-MDA5 antibody-positive patients were mainly shortness of breath upon exertion and Velcro crackles in the auscultation of bilateral lower lungs. All of these patients received a high-resolution chest CT scan. As a result, various degrees of pulmonary interstitial changes were detected in 30 patients (93.75%; Table I).

The 32 anti-MDA5 antibody-positive patients were classified into two groups: anti-Ro52 antibody-positive (n=22, 68.75%) and anti-Ro52 antibody-negative (n=10, 31.25%). The proportion of patients with RP-ILD among those with ILD in the first group was higher than in the second group (17/20, 85.0% *vs*. 2/10, 20.0%, respectively; p=0.001).

Table II. Clinical manifestations of anti-MDA5 antibody-positive patients either positive or negative for anti-Ro52 antibodies.

	MDA5 + Ro-52 (n=22)	MDA5 (n=10)	*p-value*
Interstitial lung disease / RP-ILD	20 / 17	10 / 2	0.001
Death	9	0	0.03
Subcutaneous emphysema	6	0	0.14
Hoarseness	5	0	0.16
Dysphagia	3	0	0.53

In the first group, nine patients died (40.91%); there were no deaths in the second group (p=0.03). In the first group, there were six cases of subcutaneous emphysema (27.27%), five cases of hoarseness (22.73%) and three case of dysphagia (13.64%). None of these symptoms occurred in the ten patients in the second group (Table II).

The anti-MDA5 antibody-positive patients were then divided into another two groups consisting of 21 patients diagnosed with IIM and 11 patients diagnosed with IPAF. Among the 21 patients with IIM, 18 patients (85.71%) significantly improved after treatment with prednisolone, immunosuppressive agents or immunoglobulin. Three patients died after treatment (14.29%). Among the 11 patients with an IPAF diagnosis, six patients died in spite of active treatment with prednisolone, immunosuppressive agents or immunoglobulin (54.55%; p<0.05 compared to deceased patients in the IIM group). Rapidly progressive interstitial pneumonia was more common in the IPAF group than in the IIM group (Fig. 1).

Discussion

Patients positive for anti-MDA5 antibodies are characterised by severe clinical symptoms, poor efficacy of treatment and high mortality. Thus, it is critical to improve our understanding of anti-MDA5 antibodies and their pathogenic mechanisms in order to obtain effective therapies. In this study, we summarised and analysed the clinical manifestations and outcomes of 32 anti-MDA5 antibody-positive patients, providing important clinical information to better understand this antibody.

First of all, similar to other studies (12, 13), we found that the lungs were the primary affected organ in the anti-MDA5 antibody-positive patients. Therefore, patients with inflammatory myopathy should routinely undergo high-resolution CT to evaluate lung lesions. In addition, unexplained interstitial pneumonia patients should also be given myositis-associated antibody tests as early as possible in order to facilitate disease diagnosis, treatment and assessment.

We then explored the relationship between antibodies and lung lesions. Our data showed for the first time to our knowledge that the incidence of RP-ILD, subcutaneous emphysema, hoarseness and dysphagia in both anti-MDA5 antibodies- and anti-Ro52 antibody-positive patients was significantly higher than in patients positive for only anti-MDA5 antibodies. The double antibody-positive patients also had poor prognoses with high mortality. Interestingly, similar results have been reported about anti-Ro52 antibodies. La Corte *et al*. (14) reported that patients positive for both anti-Jo-1 and anti-Ro52 antibodies had a worse prognosis in interstitial lung disease than those only positive for anti-Jo-1 antibody. Double-positive patients also experienced higher mortality. Bauhammer (15) observed that in patients with anti-Jo1 antibody, the presence of anti-Ro52 antibody was associated with severe acute-onset interstitial lung disease and non-responsiveness to immunosuppressive drugs. Based on our findings and other research, we identified anti-ro52 antibodies as an indicator of poor prognosis and a predictor of ILD progression. Although our findings require verification by large-scale studies and long-term follow-up, they indicated that doctors should be more aware of patients who are positive for both anti-MDA5 and anti-Ro52 antibodies.

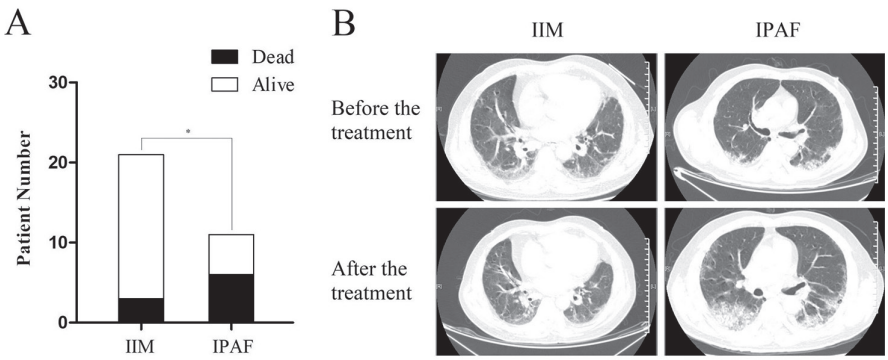

Fig. 1. **A**: Outcomes of the 32 anti-MDA5 antibody-positive patients, the dead patients in IIM group *vs*. IPAF group, **p*<0.05. **B**: CT images of the patient's lung before and after the treatment. The basic disease were idiopathic inflammatory myopathies (IIM) and interstitial pneumonia with autoimmune features (IPAF).

In this study, we found that the patients diagnosed with IIM had a relatively good prognosis, whereas patients with IPAF had a very poor prognosis. These results seemed to suggest that different basic diseases have different outcomes. We hypothesise that this may be due to the following reasons. First, anti-MDA5 antibodies are produced in different ways, and we propose that a range of factors such as connective tissue diseases, bacteria and viruses can induce their production. If anti-MDA5 antibodies are induced by connective tissue diseases, treatment with prednisolone and immunosuppressive agents may result in a relatively good prognosis. However, if the anti-MDA5 antibodies are induced by pathogens, the use of prednisolone and immunosuppressive agents is not only ineffective, but may aggravate the infection due to the immunosuppressive effect of these drugs. Recent research (16) has shown that mammalian cells rely on PRRs to detect the presence of infectious microorganisms, leading to the elimination of the infected cell via antiviral functions and apoptosis. MDA5 is one such PRR that mainly recognises viral RNAs. Therefore, anti-MDA5 antibodies would bind to the cytoplasmic PRR MDA5, blocking the response of the human body to invading pathogens. The second reason is that the anti-MDA5 antibody may exist in a variety of subtypes. Sato *et al*. (17) reported that interstitial pneumonia in anti-MDA5 antibody-positive patients could manifest as either rapid progression, chronic course or repeated relapse. Our data also indicated that although the anti-MDA5 antibody-positive patients had a high mortality, some patients greatly benefited from the treatments or had slower disease progression. Shu *et al*. (18) proposed as a possible explanation that there may be several subtypes of anti-MDA5 antibody which result in different prognoses. This explanation will require experimental confirmation.

In summary, our study increases our understanding of the anti-MDA5 antibody and provides valuable scientific information for future studies. However, due to the small sample size and relatively short follow-up time, the role of the anti-MDA5 antibody in autoimmune diseases will require further elucidation in future studies.

References

1. SATO S, HIRAKATA M, KUWANA M *et al*.: Autoantibodies to a 140-kd polypeptide, CADM-140, in Japanese patients with clinically amyopathic dermatomyositis. *Arthritis Rheum* 2005; 52: 1571-6.
2. SATO S, HOSHINO K, SATOH T *et al*.: RNA helicase encoded by melanoma differentiation-associated gene 5 is a major autoantigen in patients with clinically amyopathic dermatomyositis: Association with rapidly progressive interstitial lung disease. *Arthritis Rheum* 2009; 60: 2193-200.
3. MILAM EC, FUTRAN J, FRANKS AG Jr.: Anti-MDA5 antibody dermatomyositis overlap with systemic lupus erythematosus: a case report and review of the literature. *Open Rheumatol J* 2016; 30: 122-28.
4. YAMAZAKI K, WAKABAYASHI H, SUGANAMI

Y *et al*.: A case of dermatomyositis with severe myalgia and muscle weakness testing positive for anti-melanoma differentiation-associated gene 5 antibody. *Acta Med Okayama* 2017; 71: 341-44.
5. KATO H, TAKEUCHI O, SATO S *et al*.: Differential roles of MDA5 and RIG-I helicases in the recognition of RNA viruses. *Nature* 2006; 441: 101-5.
6. GOUBAU D, SCHLEE M, DEDDOUCHE S *et al*.: Antiviral immunity via RIG-I-mediated recognition of RNA bearing 5'-diphosphates. *Nature* 2014; 16: 372-75.
7. SONTHEIMER RD: MDA5 autoantibody-another indicator of clinical diversity in dermatomyositis. *Ann Transl Med* 2017; 5: 160.
8. ICHIYASU H, SAKAMOTO Y, YOSHIDA C *et al*.: Rapidly progressive interstitial lung disease due to anti-MDA-5 antibody-positive clinically amyopathic dermatomyositis complicated with cervical cancer: Successful treatment with direct hemoperfusion using polymyxin B-immobilized fiber column therapy. *Respir Med Case Rep* 2016; 20: 51-54.
9. CHINO H, SEKINE A, BABA T *et al*.: Radiological and pathological correlation in anti-MDA5 antibody-positive interstitial lung disease: rapidly progressive perilobular opacities and diffuse alveolar damage. *Intern Med* 2016; 55: 2241-6.
10. DALAKAS MC: Inflammatory muscle diseases. *N Engl J Med* 2015; 372: 1734-47.
11. FISCHER A, ANTONIOU KM, BROWN KK *et al*.: An official European Respiratory Society/American Thoracic Society research statement: interstitial pneumonia with autoimmune features. *Eur Respir J* 2015; 46: 976-87.
12. KOGA T, FUJIKAWA K, HORAI Y *et al*.: The diagnostic utility of anti-melanoma differentiation-associated gene 5 antibody testing for predicting the prognosis of Japanese patients with DM. *Rheumatology* (Oxford) 2012; 51: 1278-84.
13. CERIBELLI A, FREDI M, TARABORELLI M *et al*.: Prevalence and clinical significance of anti-MDA5 antibodies in European patients with polymyositis/dermatomyositis. *Clin Exp Rheumatol* 2014; 32: 891-7.
14. LA CORTE R, LO MONACO A, LOCAPUTO A, DOLZANI F, TROTTA F: In patients with antisynthetase syndrome the occurrence of anti-Ro/SSA antibodies causes a more severe interstitial lung disease. *Autoimmunity* 2006; 39: 249-53.
15. BAUHAMMER J, BLANK N, MAX R *et al*.: Rituximab in the treatment of Jo1 antibody-associated antisynthetase syndrome: anti-Ro52 positivity as a marker for severity and treatment response. *J Rheumatol* 2016; 43: 1566-74.
16. DIAS JUNIOR AG, SAMPAIO NG, REHWINKEL J: A balancing act: MDA5 in antiviral immunity and autoinflammation. *Trends Microbiol* 2018; 18: 30179-3.
17. SATO Y, OTSUKA K, TAMAI K, ONO Y, HAMAGUCHI Y, TOMII K: An atypical clinical course of anti-MDA5 antibody-positive interstitial lung disease in a patient with three deteriorations in 9 years. *Intern Med* 2017; 56: 341-46.
18. SHU E, KANOH H, MURAKAMI A, SEISHIMA M: Potential inhibition of development of rapidly progressive interstitial lung disease by prompt and sufficient immunosuppressive treatment in patients with anti-melanoma differentiation-associated gene 5 antibody-positive dermatomyositis [letter]. *J Dermatol* 2017; 44: e91-e92.