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Association of anti-HMGCR antibodies of the IgM isotype with refractory immune-mediated necrotizing myopathy

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Abstract

Objective Anti-3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMGCR) autoantibodies are one of the myositis-specific antibodies which is associated with immune-mediated necrotizing myopathy (IMNM). However, the relationship between anti-HMGCR isotypes and prognosis has not yet been fully investigated. This study was conducted to gain insight into the association between anti-HMGCR isotypes and clinical, and prognosis in IMNM patients who were positive for anti-HMGCR antibodies.

Methods Levels of anti-HMGCR isotypes (IgG, IgA and IgM) were assessed by enzyme-linked immunosorbent assay (ELISA) in 123 consecutive serum samples obtained from 71 patients who were positive for anti-HMGCR IgG at baseline. Disease activity was assessed by manual muscle testing (MMT) 8, Physician's Global Assessment (PGA) visual analog scale (VAS), and muscle VAS.

Results Baseline anti-HMGCR IgG levels were correlated with PGA VAS ($r=0.24$; $p=0.04$), muscle VAS ($r=0.32$; $p<0.01$), and MMT8(r=-0.24; $p=0.04$), and baseline anti-HMGCR IgM levels were positively correlated with PGA VAS ($r=0.27$, $p=0.02$), muscle VAS ($r=0.24$, $p=0.04$). Anti-HMGCR IgM positive patients had a lower age of onset [29(25,46) vs. 51(33,65), $p=0.006$], and a higher proportion of neck weakness (63.5% vs. 34.6%, $p=0.031$) compared with anti-HMGCR IgM negative patients. Longitudinal analysis showed that the changes in anti-HMGCR IgG levels were correlated with the changes in the PGA VAS ($\beta=3.830$; $p<0.0001$), muscle VAS ($\beta=2.893$; $p<0.0001$), MMT8 (β=-19.368; $p<0.0001$), and creatine kinase (CK) levels ($\beta=3900.05$, $p<0.0001$). Anti-HMGCR IgM levels were weakly correlated with anti-HMGCR IgA levels at baseline ($r=0.33$, $p<0.01$), and the variations in anti-HMGCR IgA levels were correlated with the changes in anti-HMGCR IgM levels during follow-up ($\beta=0.885$; $p<0.0001$). There were more patients with anti-HMGCR IgM who showed a refractory course than those who were with anti-HMGCR IgM negative (polycyclic course: 40% vs. 25%; chronic continuous course: 46.7% vs. 20.5%, $p=0.018$).

Conclusion In anti-HMGCR IgG-positive IMNM patients, the levels of anti-HMGCR IgG are associated with disease activity, and anti-HMGCR IgM is associated with refractory outcome and poor prognosis.

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Rheumatology key messages

- Anti-HMGCR IgM-positive patients had a younger age of onset and more neck weakness than anti-HMGCR IgM-negative patients.
- The levels of anti-HMGCR IgG and IgM are associated with disease activity in anti-HMGCR-positive patients.
- Anti-HMGCR IgM is associated with refractory outcome and poor prognosis.

Keywords Immune-mediated necrotizing myopathy, Anti-HMGCR, Autoantibodies isotypes, Outcome

Introduction

Anti-3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMGCR) antibodies are considered to be one of the myositis-specific antibodies (MSA) in immune-mediated necrotizing myopathy (IMNM) [1]. IMNM patients with anti-HMGCR antibodies are characterized by refractory muscle weakness and significantly elevated creatine kinase (CK) levels [2]. In daily clinical practice, the clinical phenotype and prognosis of anti-HMGCR-positive IMNM are heterogeneous. Approximately 63% of anti-HMGCR-positive patients have a history of statin exposure, and patients without a history of statin exposure have more severe muscle weakness and poorer immunosuppressive response than those with a history of statin exposure [3, 4]. In addition, younger patients with anti-HMGCR antibodies have more severe muscle weakness and slower recovery than older patients [5–8].

Anti-HMGCR antibodies can also be classified as IgG, IgA, or IgM according to their immunoglobulin isotype. In clinical practice, anti-HMGCR positivity is usually based on the presence of IgG isotype of anti-HMGCR antibodies. In previous studies, anti-HMGCR titers were correlated with muscle strength and serum CK levels, and decreases in anti-HMGCR are associated with improvements in muscle strength and decreases in CK levels after treatment [3, 9–11]. Classical complement pathway activation and in vitro experiments show that anti-HMGCR autoantibodies are pathogenic [12–14].

However, the relationship between anti-HMGCR isotypes and disease outcome has not yet been fully investigated. Therefore, we aimed to investigate the association between the presence and levels of anti-HMGCR antibodies of the IgG, IgM, and IgA isotypes and disease outcome in anti-HMGCR IgG-positive IMNM.

Patients and methods

Patients

This study enrolled 71 anti-HMGCR IgG-positive IMNM patients hospitalized at the Department of Rheumatology and Immunology of China-Japan Friendship Hospital from 2014 to 2022. The diagnosis of IMNM was made according to criteria of the European Neuromuscular Center in 2017 [1]. Anti-HMGCR IgG was tested by enzyme-linked immunosorbent assay (ELISA) (Inova Diagnostics Inc., San Diego, CA, USA) according to the manufacturer's protocol, and levels > 0.15U/ml were considered positive. Neuromuscular diseases with genetic confirmation were excluded.

In addition, 47 patients with idiopathic inflammatory myositis (IIM) who were positive for other MSA but negative for anti-HMGCR IgG and 33 age and sex-matched healthy controls (HCs) without IIM were included in this study. The diagnosis of IIM was based on the 2017 European League Against Rheumatism/American College of Rheumatology IIM criteria [15].

The demographic, clinical and laboratory characteristics of the enrolled patients were retrospectively collected from the inpatient medical record system. The diagnosis of interstitial lung diseases(ILD) was based on high-resolution chest computed tomography and abnormal pulmonary function tests [16]. Skin involvement included mechanic's hand, Gottron's sign, heliotrope sign, and Raynaud's phenomenon. Muscle atrophy was diagnosed by muscle magnetic resonance imaging. Medical Research Council (MRC) scale (grades 0–5), and Manual Muscle Testing in 8 muscles (MMT-8) were used for the muscle measurements. Severe muscle weakness was defined as a grade ≤ 3 for muscle strength on the MRC scale in any muscle at presentation [17].

Disease activity was assessed using the core set of measures developed by the International Myositis Assessment and Clinical Studies Group (IMACS) [18–20]. All patients were assessed for disease activity using the MMT-8, the Physician's Global Assessment (PGA) visual analog scale (VAS; range: 0–10 cm), and the muscle VAS.

Follow-up design

The follow-up time was defined as the interval between the diagnosis of myositis and the last follow-up. The survival status of all anti-HMGCR IgG-positive patients was obtained by telephone or medical record follow-up. All patients had at least one follow-up visit.

Patients were defined as in remission if they met the following criteria and remained in remission for at least 6 months CK < 500 U/L and MMT8 ≥ 75, and PGA VAS ≤ 1 cm. Relapse was defined as a decrease in MMT8 > 20% compared to baseline, worsening of PGA VAS ≥ 2 cm, and CK > 2000U/L or a doubling of CK levels increased compared to the previous visit [21]. Clinical courses were defined as monocyclic (complete recovery within 2 years without a relapse, with or without medical

therapy), chronic polycyclic (prolonged, relapsing course with ≥1 relapse during ≥2 years of observation), chronic continuous (persistent disease activity for >2 years, never remission during full-time follow-up despite medical therapy). Patients whose disease evolution was <1 year were not assessed for clinical outcome [22].

The study was conducted in accordance with the tenets of the Declaration of Helsinki, and was approved by the Ethics Review Committee of the China-Japan Friendship Hospital (approval number: 2019-SDZL-3). Written informed consent was obtained from all patients.

Measurement of anti-HMGCR isotypes by ELISA

The levels of anti-HMGCR isotypes (IgG, IgA and IgM) were measured by ELISA in the sera of 71 anti-HMGCR-positive patients, 47 anti-HMGCR-negative IIM patients, and 33 HCs. The levels of anti-HMGCR isotypes in the follow-up sera obtained from anti-HMGCR Ig G-positive patients were also examined. All serum samples were stored at -80℃ for the detection of anti-HMGCR antibodies.

Anti-HMGCR-IgG levels were measured by ELISA (Inova Diagnostics, Inc., San Diego, CA, USA) according to the manufacturer's protocol, using a cut-off value of 0.15 U/ml. Horseradish peroxidase (HRP)-conjugated goat anti-human IgA polyclonal antibody (1:5000; A18781; Thermo Fisher Scientific) and mouse anti-human IgM-HRP (1:4000; cat. no.9020-05; Southern Biotech) were used as secondary antibodies to test for the presence of anti-HMGCR antibody isotypes (IgA, IgM) in serum. Serum samples were diluted 1:200 for the anti-HMGCR IgA test and 1:400 for the anti-HMGCR IgM test, respectively. Antibody units were calculated from the resulting optimal densities at 450 nm (A450). The absorbance of each well was read at 450 nm and the anti-HMGCR antibody levels were calculated according to the manufacturer's instructions: unit value (U/ml) = [A450(sample)-A450(negative control)/ [A450(positive control)-A450(negative control)]. All samples were examined in duplicate, and the mean of the two assays was used as the final value for statistical analysis.

Detection of MSA

All samples were screened for anti-TIF1-γ, anti-NXP2, anti-Mi2, anti-MDA5, anti-SAE, anti-Jo-1, anti-PL-7, anti-PL-12, anti-EJ, and anti-SRP autoantibodies by line immunoassay (EUROIMMUN, Lübeck, Germany) according to manufacturer's protocol.

Statistical analysis

Categorical data are expressed as frequencies (%), whereas continuous variables are presented as mean±standard deviation(SD) if normally distributed, or median and interquartile range (IQR) if not. Comparisons between groups were made using Fisher's exact test, chi-square test, or Mann-Whitney test as appropriate. Spearman's rank correlation analysis was used to examine the association between the levels of anti-HMGCR antibodies and clinical and pathological parameters. Generalized estimating equations (GEE) were used to analyze the longitudinal correlations between anti-HMGCR levels and disease activity. Time to remission was used as an indicator to evaluate the outcome. All variables were included in the univariate analysis, and predictors with $p<0.05$ in univariable analysis were included in the multivariable Cox regression analysis. Statistical analyses were performed using SPSS version 26 (SPSS, Inc., Chicago, IL, USA) and GraphPad Prism 8.0 (GraphPad Software, San Diego, CA, USA). *P* values<0.05 were considered statistically significant.

Results

Evaluation of the isotypes of anti-HMGCR antibodies

We determined cut-off values for anti-HMGCR IgA and Ig M using the mean value of the HCs plus two SD or 3SD. When the cut-off value was set to mean+2SD (0.25U/ml for anti-HMGCR IgA and 0.41 U/ml for anti-HMGCR IgM), the positivity rates of Ig M and Ig A were 26.8% and 23.9%, respectively, and the specificity was 87.2% and 89.4%, respectively. When the cut-off value was set tomean+3SD (0.32U/ml for anti-HMGCR IgA and 0.50 U/ml for anti-HMGCR IgM), the positivity rates of Ig M and Ig A were 15.5% and 28.3%, respectively, and the specificity was 100% and 93.6%, respectively. Indeed, there is a lower positivity rate and more significant specificity when the cut-off value is set to mean+3SD (Supplementary Fig. 1 and Supplementary Table 1). Different cut-off values in our analyses did not affect the conclusions of our study. We use mean+2SD as the cut-off value in subsequent analyses.

Correlation between baseline anti-HMGCR isotypes levels and disease activity

Analysis of the correlation between baseline anti-HMGCR isotype levels and disease activity showed that baseline anti-HMGCR IgG levels were significantly positively correlated with PGA VAS ($r=024$, $p=0.04$), muscle VAS ($r=0.32$, $p<0.01$), and negatively correlated with MMT8 ($r=-0.24$, $p=0.04$) (Fig. 1A-C). Baseline anti-HMGCR IgM levels were positively correlated with PGA VAS ($r=0.27$, $p=0.02$), Muscle VAS ($r=0.24$, $p=0.04$) (Fig. 1G, H, I), while no correlation was found between anti-HMGCR IgA levels and disease activity (Fig. 1D, E, F).

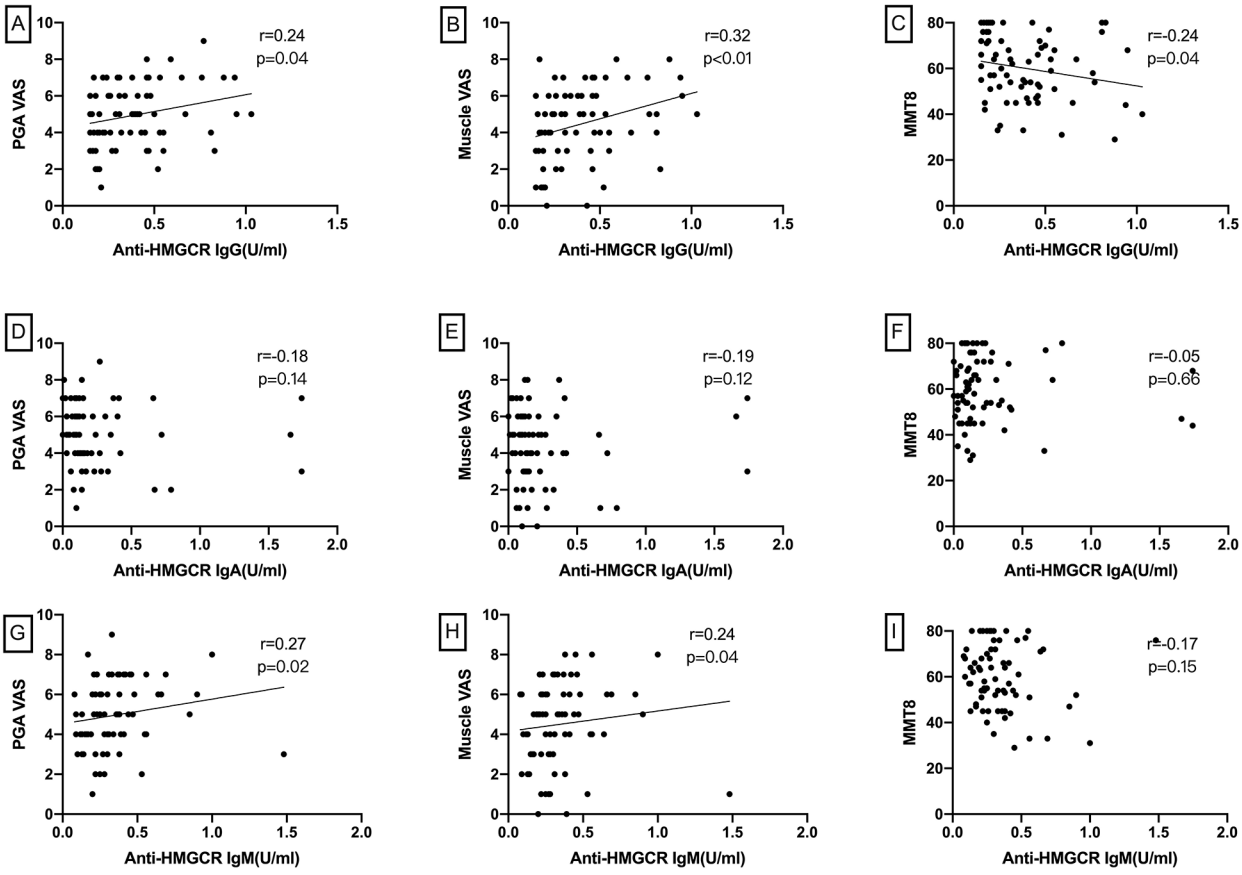

Fig. 1 Correlation between baseline anti-HMGCR Ig G (**A**-**C**), IgA(**D**-**F**), and IgM(**G**-**I**) levels and disease activity.Abbreviation: HMGCR, 3-hydroxy-3-methylglutaryl-coenzyme A reductase; PGA, Physician's Global Assessment; VAS, Visual Analog Scale; MMT, manual muscle testing

Characteristics of anti-HMGCR patients stratified by anti-HMGCR IgM phenotype

Seventy-one patients who were positive for anti-HMGCR IgG detected by ELISA were included in the study. The enrolled patients were 45 females (63.4%) and 26 males (36.6%), with an onset age of [46 (29,61; IQR)] years. The duration from onset to diagnosis was [12(5,24; IQR)] months, and 15 patients (21.1%) had a history of statin use.

Since anti-HMGCR Ig M levels were correlated with disease activity, we compared the characteristics of patients with anti-HMGCR IgM positive and anti-HMGCR IgM negative. Of the 71 anti-HMGCR IgG positive patients, 19 patients (26.7%) were also positive for anti-HMGCR IgM. Compared with anti-HMGCR IgM negative patients, anti-HMGCR IgM positive patients had a lower age of onset [29(25,46) vs. 51(33,65), $p=0.006$], and a higher proportion of neck weakness (63.5% vs. 34.6%, $p=0.031$). Meanwhile, anti-HMGCR IgM positive patients had higher levels of anti-HMGCR IgA [0.22(0.12,0.42) vs. 0.12(0.06,0.18), $p=0.006$] than anti-HMGCR IgM negative patients (Table 1). Anti-HMGCR IgM levels at baseline were weakly correlated with anti-HMGCR IgA levels ($r=0.327$, $p=0.005$), but anti-HMGCR IgG levels at baseline were not correlated with either anti-HMGCR IgA or anti-HMGCR IgM levels (Supplementary Fig. 2).

All 71 enrolled anti-HMGCR positive patients underwent muscle biopsy. When we compared the muscle pathological features of anti-HMGCR IgM positive and anti-HMGCR IgM negative patients, a higher proportion of anti-HMGCR IgM positive patients showed connective tissue hyperplasia on muscle pathology (57.9% vs. 30.8%, $p=0.037$). There were no significant differences between the two groups of patients in other pathological manifestations (Supplementary Table 2). Methods for assessing muscle pathology are presented in the Supplementary Methods.

Changes of anti-HMGCR IgG, IgA, and IgM isotypes over time

Thirty-three patients had one or more follow-up visits, 10 patients experienced a shift to negative of anti-HMGCR Ig G during follow-up, and 3 patients were anti-HMGCR IgG negative at the last visit. In contrast, 8 patients (24.2%) experienced negative-positive conversion of the

Table 1 Baseline characteristics of all IMNM patients who were positive for anti-HMGCR IgG + patients

Characteristics	All patients($N=71$)	Anti-HMGCR IgM+($n=19$)	Anti-HMGCR IgM-($n=52$)	*p* value
Female	45(63.4%)	13(68.4%)	32(61.5%)	0.594
Age of onset, median (IQR), years	46(29, 61)	29(25,46)	51(33,65)	0.006
Time from onset to diagnosis, median (IQR), months	12(5,24)	11.5(4.3,24)	12(6,28)	0.526
Statin history	15(21.1%)	2(10.5%)	13(25%)	0.186
Treatment naive	23(32.4%)	6(31.8%)	17(32.7%)	0.929
Muscle weakness	64(90.1%)	18(94.7%)	46(88.5%)	0.666
Severe muscle weakness*	51(79.7%)	16(84.2%)	32(61.5%)	0.071
Dysphagia	15(21.1%)	5(26.3%)	10(19.2%)	0.517
Neck weakness	30(42.3%)	12(63.5%)	18(34.6%)	0.031
MMT8	60(51,72)	54(44,72)	63(52,72)	0.129
Muscle atrophy	8(11.3%)	1(5.3%)	7(13.5%)	0.333
Myalgia	19(26.8%)	6(31.6%)	13(25%)	0.579
Fever	4(5.6%)	1(5.3%)	3(5.8%)	0.935
Loss of weight	20(28.2%)	4(21.1%)	16(30.8%)	0.420
Arthritis	8(11.3%)	2(10.5%)	6(11.5%)	0.905
Skin involvement	12(16.9%)	6(31.6%)	6(11.5%)	0.071
Interstitial lung diseases	13(18.3%)	2(10.5%)	11(21.2%)	0.305
Malignant tumor	3(4.2%)	2(10.5%)	1(1.9%)	0.111
Other connective tissue diseases	8(11.3%)	2(10.5%)	6(11.5%)	1
ALT, median (IQR), IU/L	123(54,224)	155(57,227)	119(47,254)	0.599
AST, median (IQR), IU/L	64(32,122)	86(29,122)	59(35,122)	0.823
LDH, median (IQR), IU/L	542(299,792)	569(296,854)	526(303,778)	0.851
CK, median (IQR), IU/L	2385(800,4790)	1812(552,4899)	2395(859,4423)	0.886
Anti-HMGCR characteristics				
IgG level, median (IQR), U/ml	0.34(0.20,0.50)	0.38(0.22,0.55)	0.33(0.20,0.48)	0.879
IgA positive(> 0.25U/ml)	17(23.5%)	8(42.1%)	9(17.3%)	0.056
IgA level, median (IQR), U/ml	0.13(0.08,0.24)	0.22(0.12,0.42)	0.12(0.06,0.18)	0.006

* severe muscle weakness was defined as a grade ≤ 3 for muscle strength in Medical Research Council scale

Abbreviations: HMGCR, 3-hydroxy-3-methylglutaryl-coenzyme A reductase; MMT, manual muscle testing; ALT, alanine aminotransferase; AST, aspartate aminotransferase; LDH, lactic dehydrogenase; CK, creatine kinase

IgM isotype of anti-HMGCR antibodies, and 11 patients (33.3%) experienced a positive conversion of IgA antibodies (Fig. 2 and Supplementary Table 3).

Longitudinal relationship between anti-HMGCR autoantibody levels and disease activity

Thirty-three patients had one or more follow-up visits. We analyzed the correlation between anti-HMGCR isotype levels and disease activity 33 patients using the GEE model. Longitudinal analysis showed that the changes in anti-HMGCR IgG levels were significantly correlated with the changes in PGA VAS (β=3.830; $p<0.0001$), muscle VAS (β=2.893; $p<0.0001$), MMT8 (β=-19.368; $p<0.0001$) and CK levels (β=3900.05, $p<0.0001$) (Supplementary Fig. 3). However, the changes in anti-HMGCR IgA and IgM levels were not associated with changes in disease activity (data not shown). Furthermore, changes in anti-HMGCR IgA levels were correlated with the changes in anti-HMGCR IgM levels (β=0.885; $p<0.0001$), but no correlation was found between levels of anti-HMGCR IgG levels and anti-HMGCR IgA or IgM levels (Supplementary Fig. 4).

We then analyzed the relationship between the changes in anti-HMGCR IgM levels and the changes in disease activity in six patients with three or more visits who had a history of anti-HMGCR IgM positivity. Four patients (P1, P2, P3, P4) were found to have progressive or persistent disease activity when anti-HMGCR IgM turned positive or remained positive, whereas no such pattern was found in some patients (P5, P6) (Fig. 3).

Outcome of patients with anti-HMGCR-positive IMNM stratified by anti-HMGCR IgM

We evaluated the outcome of anti-HMGCR IgG patients stratified by anti-HMGCR IgM profiles. The follow-up time for all 71 patients ranged from 4 to 144 months, with a median time interval of 29.4 (18.8,45.3) months. One anti-HMGCR IgM-positive woman died of a cervical malignancy 20 months after being diagnosed with myositis. Eight anti-HMGCR IgM-negative patients (16.3%) achieved remission after 6 months, but no nti-HMGCR IgM-positive patients achieved remission after half a year. More anti-HMGCR IgM-positive patients at baseline developed chronic polycyclic and chronic continuous

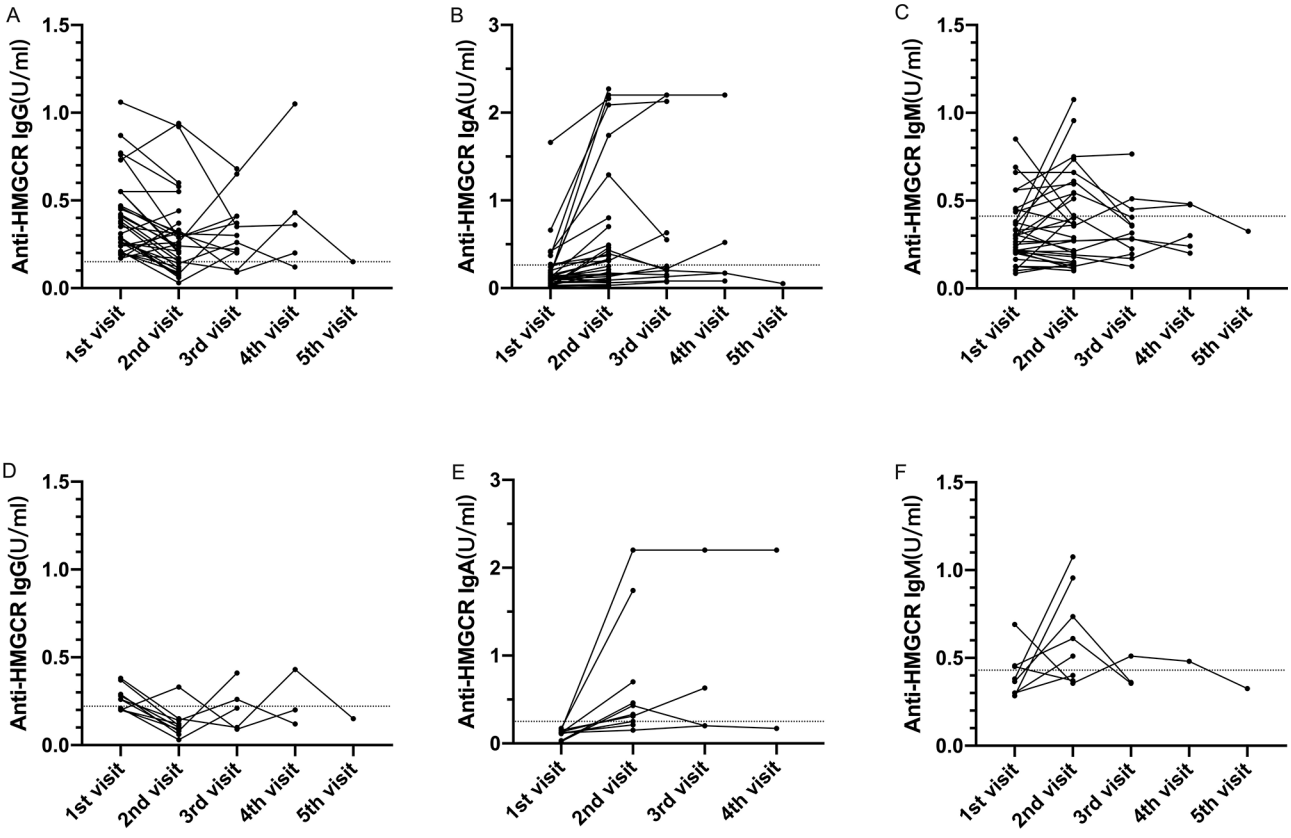

Fig. 2 Changes of anti-HMGCR IgG (**A**), IgA (**B**), and IgM (**C**) during follow-up in 33 patients with one or more follow-up visits. Changes of anti-HMGCR IgG in 10 patients with IgG negative-positive conversion(**D**), changes of anti-HMGCR IgA in 11 patients with a positive conversion of IgA(**E**), and changes of anti-HMGCR IgM in 8 patients with negative-positive conversion of the IgM (**F**) over time. Abbreviations: HMGCR, 3-hydroxy-3-methylglutaryl-coenzyme A reductase

courses than anti-HMGCR IgM-negative patients (polycyclic course: 40% vs. 25%; chronic continuous course: 46.7% vs. 20.5%, $p=0.018$) (Table 2).

We also evaluated the outcome of anti-HMGCR-positive patients stratified by anti-HMGCR IgM profiles during follow-up. There were 24 patients who developed positive for anti-HMGCR IgM antibodies during the course of the disease, and patients who were ever anti-HMGCR IgM positive also had a more refractory outcome (polycyclic course: 42.1% vs. 22.5%; chronic continuous course: 36.8% vs. 22.5%, $p=0.048$) (Supplementary Table 4).

We used time to remission as the outcome indicator, and analyzed risk factors affecting the prognosis of anti-HMGCR-positive patients using univariate and multivariate analyses. Univariate analyses showed that the age of onset, statin history, ILD, and anti-HMGCR IgM positivity were associated with clinical remission (all $p<0.05$). The pathological characteristics in Supplementary Table 2 were also included in the univariate analysis, and were not found to be associated with outcome (data not shown). Further multivariate Cox regression analysis revealed that older age of onset (HR 1.044, 95% CI 1.017–1.073, $p=0.001$), and ILD (HR 2.989, 95% CI 1.319–6.777, $p=0.009$) were favorable factors for the time to achieve remission (Table 3).

Discussion

Anti-HMGCR antibodies are autoantibodies directed against the 100kd/200kd protein identified in 2010 and are considered to be one of the serum markers for IMNM [1, 3, 4]. Anti-HMGCR IgG is the only isotype of anti-HMGCR antibodies that has been reported and studied to date. Previous studies have shown that anti-HMGCR-positive patients with statin exposure and younger age tend to have a worse prognosis [5–8]. Anti-HMGCR Ig G levels correlate with disease activity in cross-sectional and longitudinal analyses [3, 9–11]. However, the distribution, clinical association, and prognostic associations of anti-HMGCR isotypes in IMNM have not been investigated.

This study found that anti-HMGCR Ig M levels correlated with disease activity at baseline, and anti-HMGCR IgG levels were associated with disease activity in both cross-sectional and longitudinal analyses. We also found that Ig M and IgA antibodies were more likely to have

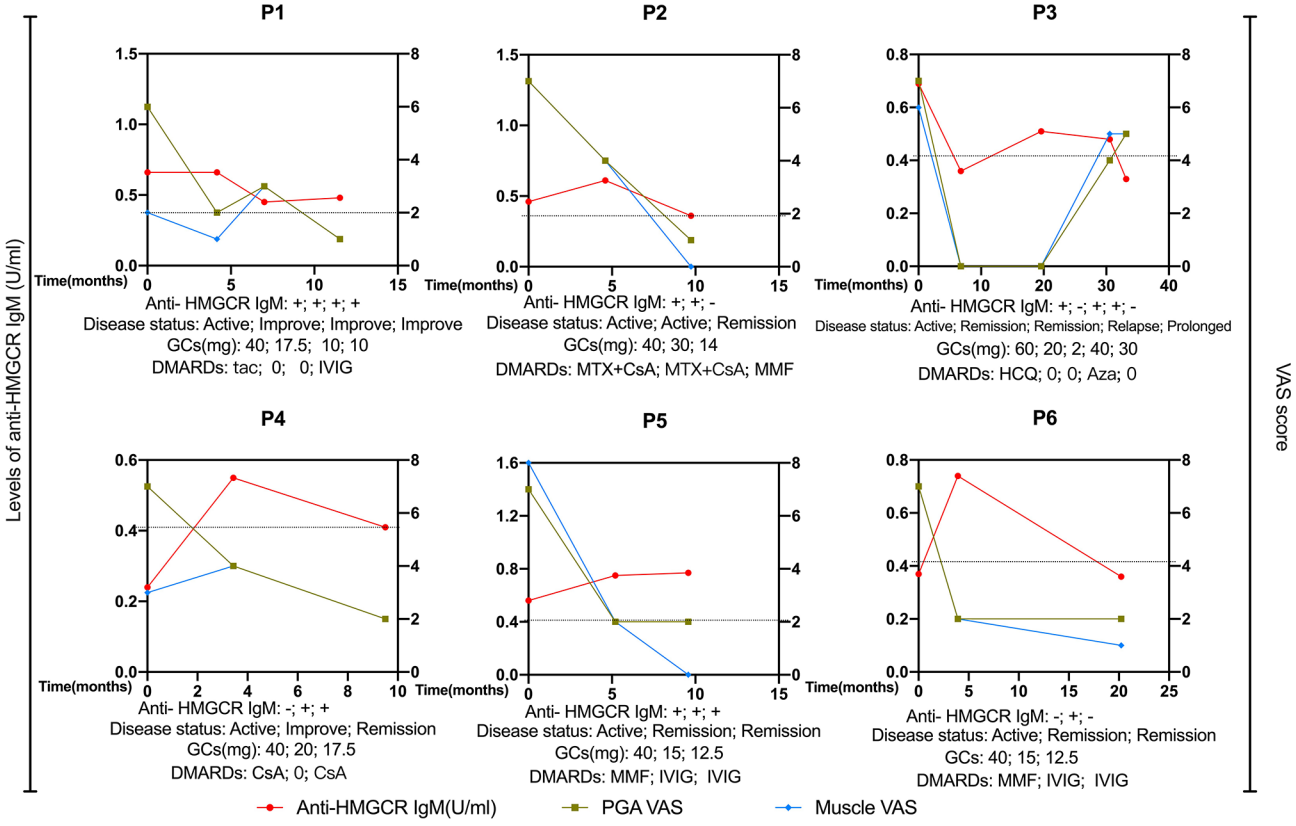

Fig. 3 Longitudinal relationship between anti-HMGCR IgM levels (red line) and PGA VAS (dark green line), muscle VAS (blue line) in six anti-HMGCR patients with three or more visits. *Abbreviations*: HMGCR, 3-hydroxy-3-methylglutaryl-coenzyme A reductase; PGA, Physician's Global Assessment; VAS, Visual Analog Scale; GCs, glucocorticosteroid; DMARDS, disease-modifying anti-rheumatic drugs; tac, tacrolimus; IVIG, intravenous. immunoglobulin; MMF, mycophenolate mofetil; CTX, cyclophosphamide; CsA, ciclosporin A; HCQ, Hydroxychloroquine

Table 2 Outcome of anti-HMGCR-positive patients stratified by anti-HMGCR IgM phenotype at baseline

Characteristics	All patients($N=71$)	Anti-HMGCR IgM + at baseline ($n=19$)	Anti-HMGCR IgM- at baseline ($n=52$)	*p* value
Follow-up time(months)	29.4(18.8,45.3)	39.4(17.2,83.8)	28.6(18.9,42.1)	0.367
Treatment				
Corticosteroids steroids	61(85.9%)	17(89.5%)	44(84.6%)	0.719
Immunosuppressive agents	38(53.3%)	12(63.2%)	26(50%)	0.432
Intravenous. Immunoglobulin	16(22.5%)	5(26.3%)	11(21.2%)	0.750
Tocilizumab	7(9.9%)	2(10.5%)	5(9.6%)	1
Outcome				
Death	1(4.1%)	1(5.3%)	0	0.268
Remission at 6 months($n=67$)	8/67(11.9%)	0/8	8/49(16.3%)	0.097
Remission at 1 year($n=66$)	23/66(34.8%)	4/18(22.2%)	19/48(39.6%)	0.187
Remission at 2 years($n=54$)	23/54(42.6%)	5/15(33.3%)	18/39(46.2%)	0.393
Relapse ($n=49$)	17/49(34.7%)	5/15(33.3%)	12/34(35.3%)	1
Clinical course				
Monocyclic	26/59(44.1%)	2/15(13.3%)	24/44(54.5%)	
Polycyclic	17/59(28.8%)	6/15(40%)	11/44(25%)	
Chronic continuous	16/59(27.1%)	7/15(46.7%)	9/44(20.5%)	0.018

a switch between positive and negative than IgG antibodies during the course of the disease, and that Ig A and Ig M levels were correlated with each other at both baseline and follow-up. Our study also found that more anti-HMGCR IgM-positive patients tended to have a refractory course.

Our study shows that anti-HMGCR IgG levels are associated with CK levels and muscle strength both at baseline and during follow-up, which is consistent with

Table 3 Univariate and multivariate cox proportional hazard models of time-to-remission

Characteristics	Remission (*n* = 35)	Non-remission (*n* = 22)	Univariate analysis		Multivariate analysis	
			HR (95%CI)	*p* value	HR (95%CI)	*p* value
Female	25(71.4%)	14(63.6%)	1.523(0.722–3.211)	0.269		
Age of onset, median (IQR), years	49(32,66)	31(18,48)	1.051(1.028,1.074)	< 0.0001	1.044(1.017–1.073)	0.001
Time from onset to diagnosis, months	12(6,19)	15(6,37)	0.992(0.977-1.007)	0.281		
Statin history	12(34.3%)	1(4.5%)	3.504(1.691–7.262)	0.001	1.744(0.750–4.059)	0.197
Muscle weakness	32(91.4%)	21(95.5%)	0.416(0.125–1.387)	0.154		
Severe muscle weakness (MRC ≤ 3)	20(57.1%)	19(86.4%)	0.586(0.298–1.154)	0.112		
Dysphagia	8(22.9%)	4(18.2%)	0.954(0.430–2.115)	0.907		
Neck weakness	13(37.1%)	10(45.5%)	1.258(0.627–2.521)	0.518		
MMT8(> 60)	17(48.6%)	8(36.4%)	1.595(0.817–3.111)	0.171		
Muscle atrophy	1(2.9%)	4(18.2%)	0.328(0.044–2.419)	0.274		
Myalgia	9(25.7%)	4(18.2%)	1.204(0.562–2.582)	0.633		
Arthritis	3(8.6%)	3(13.6%)	1.617(0.491–5.331)	0.429		
Skin involvement	7(20%)	3(13.6%)	0.778(0.337–1.798)	0.557		
Interstitial lung diseases	9(25.7%)	0	3.047(1.405–6.607)	0.005	2.989(1.319–6.777)	0.009
Malignant tumor	2(5.7%)	1(4.5%)	1.684(0.395–7.187)	0.481		
Other connective tissue diseases	3(8.6%)	2(9.1%)	0.782(0.236–2.594)	0.687		
CK, median (IQR), U/L	15(42.9%)	5(22.7%)	1.186(0.605–2.325)	0.618		
Anti-HMGCR IgG level (> 0.31U/mL)	19(54.3%)	12(54.5%)	0.667(0.339–1.312)	0.240		
Anti-HMGCR IgA positive(> 0.18U/ml)	8(22.9%)	8(36.4%)	1.085(0.477–2.470)	0.845		
Anti-HMGCR IgM positive (> 0.44U/L)	20(57.1%)	22(100%)	0.229(0.150–0.595)	0.001	1.145(0.513–2.557)	0.741

Abbreviations: HMGCR, 3-hydroxy-3-methylglutaryl-coenzyme A reductase; IQR, interquartile range; MRC, Medical Research Council; MMT, manual muscle testing; CK, creatine kinase

previous study findings [3, 9–11]. Previous studies on the pathology and pathogenesis of IMNM have shown that exposure of overexpressed HMGCR proteins on the endoplasmic reticulum induces the production of anti-HMGCR antibodies, and activates the Th1-M1-mediated immune response system, leading to necrosis, atrophy, and macrophagocytosis of muscle fibers [12–14, 23]. Immunosuppressive treatment suppresses over-activation of the immune system, followed by a decrease in anti-HMGCR IgG titers and recovery of muscle strength, which may explain the association between anti-HMGCR IgG levels and disease activity.

This study showed that persistent anti-HMGCR IgM positivity often indicates a prolonged disease course and poor response to treatment, and the occurrence of IgM positive conversion is often associated with relapse (Fig. 3; Table 2). The relationship between autoantibody isotypes and disease course and prognosis has been extensively studied in other diseases. For example, the IgM type of anti-topoisomerase I antibody is associated with disease progression in systemic sclerosis [24], and the IgM type of rheumatoid factor is associated with disease severity in rheumatoid arthritis [25, 26]. IgM is the first antibody produced after antigen exposure during the primary humoral immune response, and the presence of IgM-type antibodies often indicates recent immune activation and damage. Therefore, IgM levels of anti-HMGCR may reflect disease activity to some extent. Meanwhile, the amplified complement-killing effect of the pentameric structure of Ig M-type antibodies may also contribute to the association of anti-HMGCR Ig M with poor prognosis [27]. In addition, the antigenic epitopes recognized by anti-HMGCR IgM and IgA antibodies on muscle biopsies and their pathogenic mechanisms deserve further investigation.

In this study, we found that anti-HMGCR Ig M and IgA antibodies were more prone to antibody acquisition or disappearance during follow-up, which may be related to the fact that Ig M-type antibodies are produced first after antigen exposure, and plasma cells producing Ig M-type antibodies have a shorter lifespan [28]. We also found that the levels of anti-HMGCR IgA and IgM were correlated with each other both at baseline and during follow-up, which may be related to the class switching between Ig M, Ig G, and IgA antibodies [29].

In the analyses of prognostic risk factors, we found that younger age of onset and no ILD were risk factors for achieving remission. Previous studies have also found that younger patients have a worse prognosis in IMNM [5–8, 30].

The present study had several limitations. First, because we only included patients who were positive for anti-HMGCR IgG at baseline, we cannot exclude the possibility that some IMNM patients were only positive for anti-HMGCR IgM and/or IgA. However, the specificity of anti-HMGCR IgM/IgA was greater than 85%, regardless of the use of mean+2SD/mean+3SD as cut-off values. The effect of muscle pathology on antibody isotypes

and prognosis may require further investigation. In addition, follow-up data on antibody isotypes were available for less than half of the patients, and the findings of this study need to be validated with more follow-up data in a larger cohort.

In conclusion, this study investigated the changes of anti-HMGCR antibody isotypes during the course of the disease, and found that the presence of anti-HMGCR IgM was associated with a refractory disease course. Further research for anti-HMGCR IgM epitopes and immune response mechanisms will help to better understand the pathogenesis of anti-HMGCR-positive IMNM and find more effective treatments.

Supplementary Information

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Supplementary Material 1

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Author contributions
All authors were involved in drafting the article or revising it critically for important intellectual content, and all authors approved the final version to be published. Dr. Xin Lu had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. X. Lu were responsible for conception and design of the study. HX. Yang drafted the article or revised it critically for important intellectual content. GC. Wang, and QL. Peng contributed to acquisition and analysis of data. HX. Yang, C. Sun, LF. Ye, YT. Xu, S. Lin contributed to data analysis and interpretation.

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Data availability
Data is provided within the manuscript or supplementary information files.

Declarations

Ethics approval and consent to participate
This study was approved by the Ethics Committee of the China-Japan Friendship Hospital (2019-SDZL-3).

Consent for publication
All participants signed an informed consent agreement.

Competing interests
The authors declare no competing interests.

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