Anti-HMGCR Autoantibodies in European Patients With Autoimmune Necrotizing Myopathies

Inconstant Exposure to Statin

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Abstract: Necrotizing autoimmune myopathy (NAM) is a group of acquired myopathies characterized by prominent myofiber necrosis with little or no muscle inflammation. Recently, researchers identified autoantibodies (aAb) against 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMGCR) in patients with NAM, especially in statin-exposed patients. Here we report what is to our knowledge the first European cohort of patients with NAM.

The serum of 206 patients with suspicion of NAM was tested for detection of anti-HMGCR aAb using an addressable laser bead immunoassay. Forty-five patients were found to be anti-HMGCR positive. Their mean age was 48.9 ± 21.9 years and the group was predominantly female (73.3%). Statin exposure was recorded in 44.4% of patients. Almost all patients had a muscular deficit (97.7%), frequently severe (Medical Research Council [MRC] 5 ≤3 in 75.5%). Subacute onset (<6 mo) was noted for most of them (64.4%). Nevertheless, 3 patients (6.6%) had a slowly progressive course over more than 10 years. Except for weight loss (20%), no extramuscular sign was observed. The mean CK level was high (6941 ± 8802 IU/L) and correlated with muscle strength evaluated by manual muscle testing ($r = -0.37$, $p = 0.03$). Similarly, anti-HMGCR aAb titers were correlated with muscular strength ($r = -0.31$; $p = 0.03$) and CK level ($r = 0.45$; $p = 0.01$). Mean duration of treatment was 34.1 ± 40.8 months, and by the end of the study no patient had been able to stop treatment.

This study confirms the observation and description of anti-HMGCR aAb associated with NAM. The majority of patients were statin naive and needed prolonged treatments. Some patients had a dystrophic-like presentation. Anti-HMGR aAb titers correlated with CK levels and muscle strength, suggesting their pathogenic role.

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Abbreviations: aAb = autoantibodies, ALBIA = addressable laser bead immunoassay, AU = arbitrary units, C5b–9 = membranolytic attack complex, CK = creatine kinase, DMARD = disease-modifying antirheumatic drugs, HMGCR = 3-hydroxy-3-methylglutaryl-coenzyme A reductase, IVIg = intravenous immunoglobulin, MHC = major histocompatibility complex class I antigen up-regulation, MMT = manual muscular testing, MRC = Medical Research Council, NAM = necrotizing autoimmune myopathy, SRP = signal recognition particle.

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INTRODUCTION

Necrotizing myopathies are characterized by predominant muscle fiber necrosis and regeneration with little or no inflammation. Among the inflammatory myopathies, this histologic pattern defined a new subgroup called immune-mediated necrotizing myopathy[7] or necrotizing autoimmune myopathy (NAM). The immune-mediated nature of these myopathies was first suggested by their response to immunosuppressive treatments[4,8,14] and their frequent association with anti-signal recognition particle (anti-SRP) autoantibodies (aAb).[8,14] Nevertheless, anti-SRP aAb are observed in only 16% of necrotizing myopathies.[5] Remarkably, Mammen and colleagues[5,17] recently identified a new specific autoantibody recognizing 200 and 100 kDa proteins, initially in 16 United States patients (61%) with pathologic features of necrotizing myopathy without any known myositis-specific antibody. This United States cohort was then expanded by 29 patients (n = 45) in whom the 100 kDa protein was identified as the target of statins 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMGCR).[9] Anti-HMGCR-positive (Anti-HMGCR+) patients were in their fifth decade of life[9,11] and were very frequently exposed to statin (72.7% of cases).[11] They presented proximal and symmetric muscular deficit with high creatine kinase (CK) levels (mean, 9718 ± 7383 IU/L) and responded to immunosuppressants.[9] Since anti-HMGCR aAb were not observed in a large cohort of statin-exposed patients, including patients with self-limited statin intolerance,[10] anti-HMGCR aAb are considered specific for NAM.[10] To validate this concept in a different cohort of patients by using an alternative independent testing assay, we report herein a second cohort, equivalent in size, of anti-HMGCR+ patients with their clinico-pathologic features.

METHODS

Anti-HMGCR Detection and Quantitative Titration

For anti-HMGCR aAb detection and quantification of serial titers, we recently developed a new addressable laser bead immunoassay (ALBIA), using the same strategy as that previously used for anti-SRP aAb quantification.[2] The technical description and diagnostic value of this assay are detailed elsewhere.[5a] Briefly, the catalytic domain of recombinant human HMGCR protein fused to a glutathione S transferase tag was obtained from Sigma (Saint Louis, MO) and coupled to Bio-PlexR COOH beads with the Bio-PlexR amine coupling kit according to the manufacturer's protocol (Bio-Rad, Hercules, CA); 1250 HMGCR-coated beads were added to 100 μL of patient or control serum for 2 hours under shaking. Biotinylated mouse anti-human IgG-specific secondary Ab (Southern Biotech, Birmingham, AL) was added, and, after washing, beads were further incubated with streptavidin-R-phycoerythrin (Qiagen). Blank (no serum, secondary antibody only), negative controls (anti-HMGCR Ab-negative serum), and positive controls (highly positive human anti-HMGCR Ab serum) were included in every assay. Mean fluorescence intensity (MFI) was determined on a Bio-Plex apparatus using the Bio-Plex Manager Software 4.0 (Bio-Rad Laboratories, Hercules, CA). Anti-HMGCR Ab titers were determined at a 1/D dilution using the following formula: [MFI serum/MFI calibrator] × [titer of calibrator] × D/500. The calibrator is a highly positive human anti-HMGCR Ab serum (the same throughout the study) whose titer was arbitrarily set to 100 arbitrary units (AU/mL). We tested the first 37 sera that were found positive with this assay, and all 37 sera also scored positive for anti-HMGCR aAb using the recently developed Myositis Euroline 7 line immunoassay from Euroimmun (Lübeck, Germany). The assay was always negative when testing control sera from healthy blood donors (n = 100) or from patients with different inflammatory/autoimmune diseases (n = 142), according to established classification criteria: American College of Rheumatology revised criteria for systemic lupus erythematosus[13] with anti-dsDNA aAb, American Rheumatism Association criteria for rheumatoid arthritis[1] with anti-CCP antibodies and/or rheumatoid factor, revised European criteria for primary Sjögren syndrome[16] with anti-SSA and/or anti-SSB aAb, Bohan and Peter criteria[3] for dermatomyositis, Troyanov criteria for overlap myositis[15] with anti-tRNA-synthetase Ab, and Griggs criteria for inclusion-body myositis.[6]

Patients and Data Collection

We tested first the sera of 23 patients having the diagnosis of NAM based on clinical and histologic criteria. These patients were recruited by clinicians (YA, AR, AB, PL, TS, BE, SH, and OB) from the neuromuscular diseases reference center of East Paris. The positive first results were communicated to the French Myositis Network. Sera of 183 patients (including 8 pediatric patients) selected by clinicians of the network were sent to what was until June 2013 the sole French immunology laboratory able to detect anti-HMGCR. These 183 patients had a suspicion of NAM based on clinical and/or pathologic criteria. All these patients were also selected because of their negativity for myositis-specific aAb anti-Jo-1 and anti-SRP using a commercial kit (dTek or Euroimmun). Furthermore, all patients diagnosed as anti-HMGCR aAb positive were also tested for the presence of the following myositis-specific aAb: anti-PL-7, anti-PL-12, anti-SRP, anti-PMSCl, and anti-Mi2 (using a commercial kit, either dTek or Euroimmun). We controlled negativity for anti-SRP aAb detection using analytical sensitivity and specificity of ALBIA–SRP.[2]

All patients determined to be anti-HMGCR aAb positive by ALBIA-HMGCR from July 2012 to June 2013 were recorded for this study. For all patients, the following parameters were recorded: age, sex, past medical history, statin exposure, characteristics of myopathy including date of diagnosis, clinical manifestation with muscular deficit evaluation (by muscle manual testing [MMT] using the Medical Research Council [MRC] scale on 5 points), CK level, and reports of muscle biopsy.

Biopsies of 20 patients were performed following standardized procedures and were analyzed by the same pathologists exclusively specialized in muscle pathology (OD and TM) permitting semiquantitative analyses and standardized immunohistochemistry analyses. In those cases, muscle tissue was frozen and stored at −80°C. On 8 μm-thick cryosections, morphologic analysis (hematin and eosin counter staining) and immunohistochemistry analyses were performed. Features such as the presence of necrosis/regeneration fibers, inflammation, major histocompatibility complex (MHC) class I antigen up-regulation, membranolytic attack complex (C5b–9) deposits and vacuoles were recorded.

First-line treatments including corticosteroid and disease-modifying anti-rheumatic drugs (DMARD) were recorded as well as treatment intensification during the follow-up. Treatment intensification was defined as an introduction or an increase of more than 50% of the dose of corticosteroids and/or the introduction of DMARD.

TABLE 1. Characteristics of 45 Anti-HMGCR+ Patients

Characteristic	
Age, yr; mean	48.9 ± 21.9
Sex ratio (M:F)	0.36
Statin exposure	44.4% (n = 20)
Muscular involvement	
Muscular deficit	97.7% (n = 44)
Subacute onset	64.4% (n = 29)
Progressive onset	33.3% (n = 15)
Severe deficit (≤3)	75.5% (n = 34)
Myalgia	53.3% (n = 24)
Dysphagia	26.7% (n = 12)
CK level	6941 ± 8802 IU/L
Extraskeletal muscular involvement	
Weight loss	20% (n = 9)
Interstitial lung disease	2.2% (n = 1)
Cardiac insufficiency	2.2% (n = 1)
Arthralgia	11.1% (n = 5)
Raynaud phenomenon	11.1% (n = 5)

For this medical research where we used identifiable human sera and data, we obtained agreement from the French Ministry of Research (AC 2008-87) for the collection, analysis, storage, and reuse. All samples were obtained for diagnostic purposes. In this situation of retrospective study, patients' consents were impossible or impractical to obtain. We conducted the research after consideration and approval of the research ethics committee of Pitié-Salpêtrière Hospital.

Statistical Analysis

Categorical variables are reported as numbers and/or percentages and were compared using a chi-square or, when appropriate, Fisher exact test. Quantitative variables are reported as mean (± standard deviation) and compared using a nonparametric test. For all statistical analyses, $p < 0.05$ was considered significant. Correlation analysis was performed using the Spearman test. Statistical analyses were conducted using Prism software.

RESULTS

Characteristics and Statin Exposure in Anti-HMGCR+ Patients

Forty-five anti-HMGCR+ patients, coming from 17 different French hospitals in the myositis network, were included in the study. This represented 21.8% of the total of tested patients (n = 45/206), 26% of the first series of patients (n = 6/23), and 21.3% of the second series of patients (n = 39/183). Most patients were white, except 3 African and 1 Asian patients. Patients had a mean age of 48.9 ± 21.9 years at the time of the first signs of the disease. They were predominantly female (n = 33/45; 73%) (Table 1). One patient had onset of the disease at 3 months of pregnancy, and a second at immediate childbirth.

Eight pediatric cases (age range, 4-16 yr) were observed. Of note, only 1 patient among the 8 was tested and found to be anti-HMGCR+ before the age of 16 years. For the 7 others, the diagnosis was performed in adulthood. Four girls were initially diagnosed as having an inflammatory myopathy (polymyositis, n = 1; dermatomyositis, n = 2; NAM, n = 1) because of rapid progressive scapular and pelvic deficit with high CK level. Four other young girls were initially diagnosed as having limb girdle muscular dystrophy (LGMD) because of a slowly progressive muscle deficit.

Autoimmune diseases were noted in the past medical history of 5 patients (thyroiditis, n = 4, and type I diabetes, n = 1).

Statin exposure was present in 20 (44.4%) patients. These patients received mainly atorvastatin (n = 10/20) and rosuvastatin (n = 4/20). Statin-exposed patients were older than statin-naive patients (mean age, 64.4 ± 6.8 yr vs. 36.6 ± 21.7 yr, respectively; $p = 0.001$).

Muscular Deficit and CK Level in Anti-HMGCR+ Patients

The circumstances of discovery of NAM were mainly signs related to muscle weakness (n = 37). Otherwise, 3 patients declared having isolated myalgia, and 4 consulted for increased CK level without any other symptoms. For 1 patient, anti-HMGCR presence was suggested by a cytoplasmic fluorescence pattern on antinuclear screening test for the etiology of deep venous thrombosis.

During the course of the disease, with a mean follow-up of 49 ± 22 months, 53.3% of patients (n = 24/45) suffered from myalgia, and 97.7% of patients (n = 44/45) had a muscle weakness. Subacute onset (<6 mo) was noted for most of them (see Table 1); nevertheless, 33.3% of patients (n = 15/45) had a slow progressive muscular deficit ranging from 10 months to years. Of note, 3 cases had been considered to be muscular dystrophy for more than 10 years prior to the detection of anti-HMGCR aAb.

In most of the cases (n = 34/45, 75.5%), MMT showed a severe proximal deficit (MRC score ≤3/5 for the weakest muscular group), and 5 patients were bedridden at diagnosis (see Table 1). Axial weakness and dysphagia were found in 35.5% (n = 16/45) and 26.7% (n = 12/45) of patients, respectively. No facial deficit was observed. Marked muscle atrophy was noted for 22.2% of patients (n = 10/45), and 2 patients also had scapular winging.

All patients had increased CK levels (mean, 6941 ± 8802 IU/L). Furthermore, CK level correlated with muscular strength ($r = -0.37$, $p = 0.03$) according to the weakest muscle groups (Figure 1).

Except for the onset age, no significant difference was observed in statin-exposed patients versus nonexposed patients

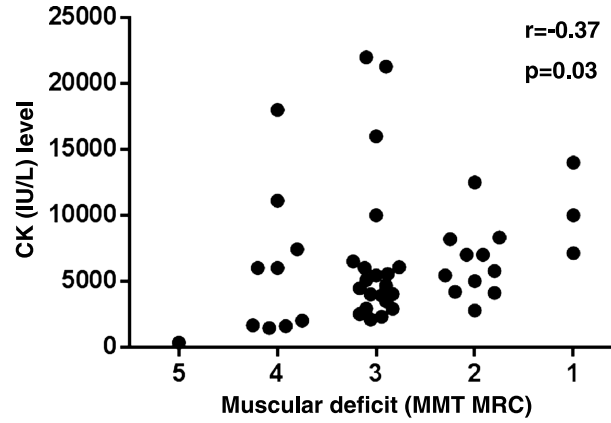

FIGURE 1. Correlation between CK level and muscular deficit in patients with NAM. For each patient the muscular deficit is given as the Medical Research Council score of the weakest muscular group using manual muscular testing (MMT MRC).

concerning percentage of myalgia, severity of muscular deficit, percentage of dysphagia, and mean CK level (data not shown).

Histologic Necrotizing Myopathy Pattern

All but 2 patients had a muscle biopsy (n = 43) performed for the diagnosis of the myopathy. Morphologic analysis showed for 42 patients (97.6%) a necrotizing myopathy pattern defined by the presence of fibers with necrosis and/or regeneration and no or few inflammatory infiltrates.

Results of 20 muscular biopsies performed in our reference center for neuromuscular disorders following standardized procedures and analyzed by the same myo-pathologists are represented in Table 2. Necrosis and/or regenerative phenomena varied from 1 patient to another, ranging from few to intense muscular necrosis (see Table 2 and Figure 2A and B). This pattern was associated with irregularity of the size of fibers in 90% of cases (n = 18/20) due to presence of atrophic fibers with nonspecific repartition, notably in the perimysium (see Table 2 and Figure 2C). Most patients (60%, n = 12/20) did not have muscular infiltrates. Six patients had small muscle infiltrates, and only 2 had mild (but obvious) inflammatory infiltrates (see Figure 2C).

C5b9 deposit was frequently observed on necrotic fibers but also at the membrane of few normal fibers (65%, n = 13/20) (see Table 2 and Figure 2D). Deposits of C5b9 decorating a few muscle capillaries were occasionally observed (25%; n = 5/20) but not as dramatically as is typically seen in dermatomyositis.

Inflammation, when present, was usually in perivascular areas. Overexpression of MHC class I by fibers was mostly observed on regenerative or necrotic fibers (Figure 2E). Nevertheless, sometimes (n = 8) a few normal fibers, in focal area, had MHC class I overexpression. Only 2 patients (including the 1 with the most obvious inflammatory infiltrates) had diffuse and intense MHC class I overexpression (Figure 2F), as can be observed in polymyositis or inclusion body myositis.

Extramuscular Involvement

Some patients (20%; n = 9/45) had fatigue associated with weight loss (range, 2–18 kg). Twenty-one percent of patients (n = 6/28) had increased C-reactive protein serum levels (range, 6–79 mg/mL). Among the 27 patients who had a computed thoracic scan, none had an interstitial lung disease, except 1 patient in whom an idiopathic interstitial lung disease was diagnosed by a lung biopsy 8 years before the onset of the myopathy. Among the 27 patients who had pulmonary function tests, 7 (25.9%)

TABLE 2. Histologic Muscle Analysis*

Patient	Fiber Necrosis and Regeneration	Disparity of Fiber Size	Muscular Infiltrates	Topography of Infiltrates	C5b9 Deposits	Diffuse Fiber MHCI Overexpression	Endomysial Fibrosis	Rimmed Vacuoles
1	++	Yes	−	−	Capillaries and fibers	No	No	No
2	++	Yes	+	Pericapillary and perinecrotic	Fibers	No	No	No
3	+	No	−	−	−	No	No	No
4	+++	Yes	+	Pericapillary	−	No	Yes	No
5	+	No	−	−	Fibers	No	No	No
6	+	Yes	−	−	−	No	No	No
7	-	Yes	−	−	−	No	No	No
8	++	Yes	−	−	Capillaries and fibers	No	No	No
9	+++	Yes	+	Pericapillary	Capillaries and fibers	No	No	No
10	++	Yes	−	−	−	No	Yes	No
11	++	Yes	+	Pericapillary	−	No	No	No
12	+++	Yes	++	Endomysial and pericapillary	Fibers	Yes	Yes	Yes
13	++	Yes	++	Endomysial and pericapillary	Fibers	No	No	No
14	++	Yes	−	−	Fibers	No	Yes	No
15	+	Yes	+	Pericapillary	−	No	No	No
16	++	Yes	−	−	Fibers	No	No	No
17	+++	Yes	+	Endomysial	Capillaries and fibers	No	No	No
18	+++	Yes	−	−	Fibers	Yes	No	Yes
19	+++	Yes	−	−	Capillaries and fibers	No	No	No
20	+++	Yes	−	−	Fibers	No	No	No

*Table shows the results of 20 patients with muscular biopsy following the same standardized procedures performed in 1 center. Semiquantitative analysis of muscular necrosis and regeneration were performed (+ corresponds to <10 necrosis and/or regenerative fibers, ++ corresponds to 10–30, and +++ corresponds to >30 per biopsy). C5b9 deposits were considered only on non-necrotic fibers or capillaries.

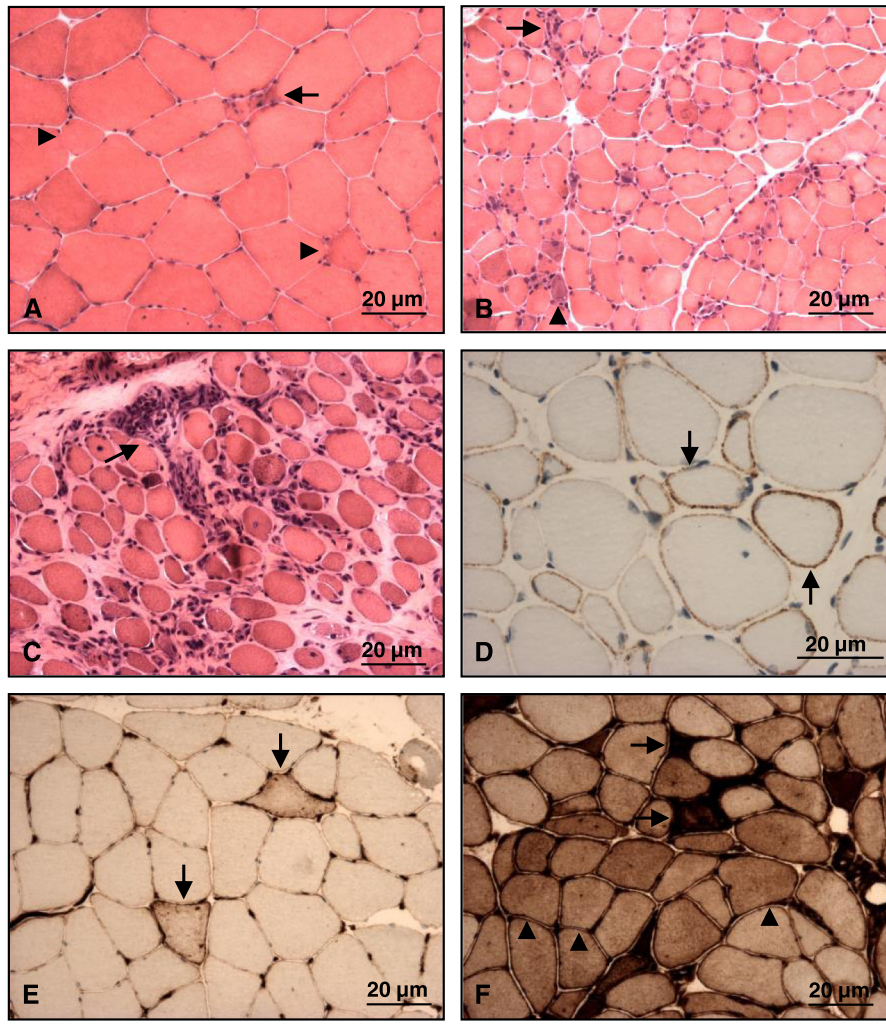

FIGURE 2. Histologic and immunohistologic analysis of muscle biopsies from anti-HMGCR+ patients. A, Muscle biopsy from a NAM patient showing rare necrotizing fiber (arrow) with some atrophic fibers (arrowheads) (hematin eosin stain). B, Muscle biopsy from another patient showing a high number of necrotized fibers (arrow) with important numbers of regenerative fibers (arrowhead). C, Muscular infiltrates are sometimes present (arrow) and in low intensity as represented here (arrow) for 1 patient. D, Immunohistologic analysis showing C5b9 deposits decorating some necrotic and non-necrotic fibers (arrows). E, Immunohistologic analysis of MHC class I expression showing a representative case of MHC overexpression on 2 fibers (arrows). F, A few patients may have diffuse and intense MHC overexpression not only on necrotic fibers (arrows) but also on non-necrotic fibers (arrowheads).

had a restrictive syndrome with a decrease of forced vital capacity (range, 33%–42% of predicted volumes).

On electrocardiogram, 4 patients (12.9%; n = 4/31) had conduction abnormalities (right branch block, n = 2; left hemi branch block, n = 2). One patient had an auricular fibrillation, and 1 an atrial flutter. For these 2 patients, 1 (aged 72 yr) was diagnosed 3 years before the onset of the myopathy and the other in the context of venous thromboembolism. Among 25 patients who had an echocardiogram, only 1 showed a decrease of ejection fraction (measured at 20% of normal); this patient was known to have an idiopathic dilated cardiomyopathy for more than 20 years before the onset of the skeletal myopathy. Seven patients had Raynaud phenomenon (n = 5/45) or acrosyndrome (n = 2/45), and 5 patients had arthralgia without synovitis. No patient had any association with another connective tissue disease, nor presented other specific aAb.

Five patients had cancer during their medical history. The cancers were diagnosed 19 years (breast adenocarcinoma), 4 years (breast adenocarcinoma), 12 months (melanoma), 6 months (renal adenocarcinoma), and 2 months (pulmonary adenocarcinoma) after the first signs of the NAM.

Treatment and Evolution

Thirty-nine of the 45 patients (86.6%) received immunosuppressive drugs and/or immune-modulatory treatment. Six patients (statin-exposed, n = 4) were not treated. One non-statin-exposed patient without muscular deficit was not treated, but follow-up time by the end of the study was less than 6 months. Two patients refused the treatment. Three remaining statin-exposed patients did not receive any treatment. They slowly improved after statin withdrawal without any other intervention. One had complete remission (normal strength and CK level) 8 months after statin withdrawal, and because she had severe familial hypercholesterolemia, statins were reintroduced without any muscular symptom or CK increase. Another patient improved in strength (with a MRC score at 4 vs. 3 concerning the weakest muscular group) and decreased CK level (500 IU/L vs.

TABLE 3. Treatments Used for NAM Patients

Patient Number	First-Line Treatment	Number of Treatment Intensification	Disease-Modifying Antirheumatic Drugs	Treatment Duration (Months)
2	CT + IVIg + MTX	0	none	3
3	CT + MTX	0	none	1
4	MTX + CT	0	none	3
6	CT + MTX + PE + IVIg	1	CT/MTX/RTX	3
7	CT + MTX	0	none	3
8	CT + MTX	2	CT/MMF/TACRO/PE/MTX/IVIg	24
9	CT + MTX + PE + IVIg	0	none	18
11	CT	5	CT/AZA/MTX/CYC/MMF/IVIg	114
12	CT + IVIg	4	CT/AZA/MTX/RTX/PE/IVIg	41
13	CT + MTX	4	CT/IVIg/RTX	42
14	CT + IVIg + MTX	2	CT/AZA/IVIg/RTX	40
16	CT + IVIg	4	CT/MTX/IVIg/MMF/RTX	62
17	CT + MTX	1	CT/MTX	36
18	CT + MTX	0	none	4
19	CT + IVIg + MTX	0	none	1
20	CT + EDX	0	none	3
21	CT + MTX	3	CT/AZA	80
22	CT	5	CT/IVIg/AZA/MTX/RTX	180
23	CT	6	CT/IVIg/MTX/AZA/MMF	90
24	CT	1	CT/AZA	34
25	CT	10	CT/IVIg/AZA/CYC/TACRO/RTX	120
26	CT + MTX	1	IVIg	18
27	CT	1	CT/IVIg/PE/MTX	5
28	CT + IVIg + MTX	2	IVIg	8
29	CT	2	MTX/PE/IVIg/RTX	11
30	CT + IVIg	1	CT/EDX	23
31	CT	1	IVIg	12
32	IVIg	0	none	7
33	IVIg	0	none	7
34	CT + MTX	3	CT/MTX/IVIg/MMF/RTX/AZA	42
35	CT	3	CT/MMF	89
36	CT	3	CT/EDX/IVIg	36
37	CT + MTX + IVIg	0	CT/MTX/IVIg	4
38	CT + MTX	1	CT/MTX/IVIg	3
39	CT	1	IVIg	50
40	CT	1	CT/AZA	6
41	CT	1	MTX	84
42	CT + MTX	0	none	11
43	CT + MTX	1	CT/MTX	12

Abbreviations: AZA = azathioprine, CT = corticosteroid, CYC = cyclosporine, EDX = cyclophosphamide, IVIg = intravenous immunoglobulin, MMF = mycophenolate mofetil, MTX = methotrexate, PE = plasmapheresis, RTX = rituximab, TACRO = tacrolimus.

5000 IU/L) at month 4. The remaining patient was diagnosed 18 months after statin withdrawal. At this period, muscle strength was 3 for the weakest muscular group and CK level was 500 IU/mL, whereas it was 2500 IU/mL 18 months before. Four months later, the patient's strength spontaneously increased from 3 to 4, myalgia disappeared, and CK level decreased to 240 IU/L.

For the other statin-exposed patients (n = 16), statin was removed and immunosuppressive drugs were initiated. Of note for 2 patients, statin was reintroduced without significant flare. Mean duration of treatment for 39 treated patients was 34.1 ± 40.8 months (range, 1–180 mo). By the end of the study, no patient had been able to stop the treatment for a prolonged time over 1 year because of relapse.

For the first line of treatment all patients but 2 were treated with corticosteroids, frequently associated with immunosuppressive drugs (methotrexate, n = 20; cyclophosphamide, n = 1) or intravenous immunoglobulin (IVIg, n = 10, Table 3). One bedridden patient was also treated by plasmapheresis as first-line treatment.

All patients but 1 who were treated for more than 1 year required intensification by DMARD (see Table 3). Because of flares or insufficient control of the NAM, the mean number of treatment intensifications was 1.8 ± 2.1 (range, 1–10).

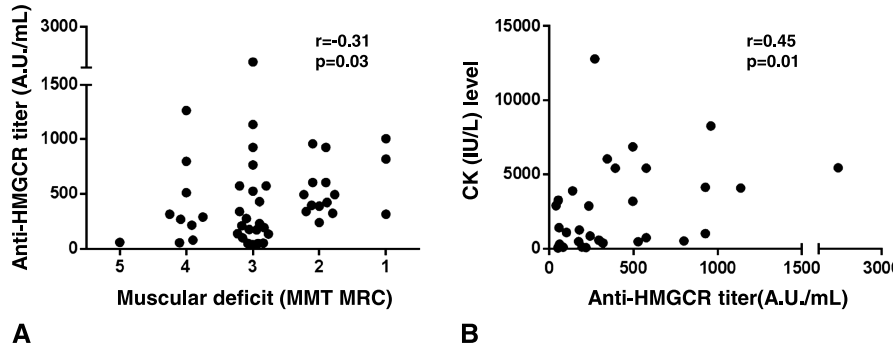

FIGURE 3. Correlation between muscular deficit and CK level with anti-HMGCR aAb titer. For each patient, we analyzed the correlation between the anti-HMGCR aAb titer and muscular deficit given as the MRC score of the weakest muscular group using manual muscular testing (MMT MRC) (A) or CK level (B).

DMARD were methotrexate (n = 15), azathioprine (n = 10), mycophenolate mofetil (n = 6), cyclosporine (n = 2), tacrolimus (n = 2), cyclophosphamide (n = 2), rituximab (n = 9), plasmapheresis (n = 3), IVIg (n = 17). The mean treatment duration and the number of treatment intensifications was not significantly different between statin-exposed and statin-naive patients (32.2 ± 39.4 mo vs. 30.8 ± 32.3, respectively; p = 0.83; and 1.9 ± 1.8 vs. 1.1 ± 1.5, respectively; p = 0.31).

By the end of the study only 1 patient had died, due to aspiration pneumonia; this patient was bedridden.

Titer of Anti-HMGCR

Finally, in an attempt to indirectly investigate a possible physiopathogenic role of anti-HMGCR, we looked for correlation between aAb titer and the muscular parameters: strength and CK level. We observed a significant correlation (r = −0.31; p = 0.03) between the anti-HMGCR aAb titer and the MRC score of the weakest muscle groups (Figure 3A). Similarly, we observed a significant correlation (r = 0.45; p = 0.01) between the aAb titers and CK level tested the same day (Figure 3B).

Of note, no significant difference was observed between HMGCR titer in statin-exposed and statin-naive patients (data not shown). Differentiating statin-exposed and statin-naive patients, we did not observe a significant correlation between CK levels and aAb titers (r = 0.6; p = 0.06 and r = 0.38; p = 0.09, respectively). But a significant correlation between muscle strength and aAb titers was observed in the statin-naive group (r = 0.56; p = 0.01), which we did not observe in the statin-exposed group (r = 0.06; p = 0.6).

DISCUSSION

In this study describing the second case series of anti-HMGCR NAM patients (n = 45), we found that both children and adults may be affected by a severe muscular deficit with an acute or a more slowly progressive onset, without extramuscular involvement in most cases. Most patients required several lines of treatment over a prolonged period. The high CK level correlates with muscular strength, and the titer of anti-HMGCR aAb correlates with muscular strength and CK level.

We observed a marked female predominance (73%) that the previous study did not observe (57.8%).[9] Of note, the onset of disease in 2 patients (5%) was during or just after pregnancy, which is a rare situation in other forms of autoimmune myopathies.[11] The 2 newborns did not present any sign of myopathy.

Eight of the 45 cases involved patients aged younger than 16 years. This observation and the 2 pediatric cases reported by Mammen et al[9] suggest that anti-HMGCR NAM should be considered in the differential diagnosis in children with myopathy, as was previously shown for anti-SRP NAM.[12] Dermatomyositis is not the sole inflammatory myopathy occurring during childhood.

We observed that the onset of the muscular deficit in anti-HMGCR+ patients may be slow, and 3 patients had a disease progression for more than 10 years before the diagnosis. Typically, these patients were initially considered to have a limb girdle muscular dystrophy with no molecular diagnosis. Indeed, muscle histologic analysis may show a dystrophic pattern with necrosis/regeneration, associated with irregular size of fibers and endomysial fibrosis. This pitfall was also described in anti-SRP NAM.[2] It led us and others[12] to recommend testing for anti-SRP and anti-HMGCR aAb in patients presenting clinical and pathologic features compatible with limb girdle muscular dystrophy with no molecular diagnosis.

Of note, muscular inflammation may also be observed on muscle biopsy. Yet, our cohort of anti-HMGCR+ subjects did not include patients with prominent inflammatory cell infiltrates, since anti-HMGCR aAb were only looked for in patients suspected of having NAM and, presumably, patients with significant degrees of inflammation were not screened for anti-HMGCR antibodies.

Contrary to the case of other overlap syndromes associated with myositis-specific antibodies, such as antisynthetases, we did not observe clear extramuscular involvement in anti-HMGCR+ patients. For example, no patient had interstitial lung disease. This is in line with the observations of Mammen et al.[9]

Only 44% of patients were statin exposed in the current study, compared to the 72.7% frequency reported in the study by Mammen et al.[17] While we also observed that statin-naive patients were younger, we did not find any other difference between the statin-naive and statin-exposed groups, whereas Mammen et al[9] found that mean CK levels were lower in statin-exposed patients. The difference in the prevalence of statin exposure in our study compared to Mammen's study may be explained by the fact that we studied a different population. For example, our study is a collaborative work involving 2 pediatric and 2 dystrophic centers. Thus, our cohort is younger on average (aged 44 ± 19 vs. 52 ± 16 yr) and includes subjects who were initially considered to have an inherited myopathy. Another difference is that Mammen and colleagues[9] screened their entire cohort of subjects suffering from

inflammatory myopathies for anti-HMGCR antibodies, whereas we screened mainly those suspected of having NAM.

Three statin-exposed patients improved within months after statin withdrawal without any other intervention. Such cases were not previously described. It may suggest that some muscular statin-intolerant patients are in fact real NAM cases. Mammen et al[10] tried to address this point and showed that none of 51 patients with self-limited statin intolerance was anti-HMGCR aAb positive. Nevertheless, anti-HMGCR+ NAM diagnosis cannot be ruled out in patients diagnosed as "statin-intolerant" especially if a high CK level is observed. This difference may be due to the technology used for the detection of anti-HMGCR aAb and/or patient recruitment, since statin-intolerant had a mild increase in CK level (131.44 ± 71.07 IU/L) as compared to our 3 patients with CK level above 1000 IU/L.

Anti-HMGCR treated patients had long treatment duration, in line with those previously reported.[17] At the study's end, all patients continued their treatment and almost everyone required DMARD (ranging from 1 to 10) for intensification, suggesting that anti-HMGCR NAM is a severe disease justifying prolonged treatment.

The correlation between CK level and muscular strength suggests that CK level monitoring may be a good surrogate biomarker of disease activity. Nevertheless, the correlation coefficient was not high. This is not surprising since other parameters may be involved in muscular deficit, such as uncontrolled disease duration and/or muscular adipose involution.

We also confirmed what Mammen and colleagues[17] first reported, that anti-HMGCR titers correlated with muscle strength and CK levels. Together, these results argue for a possible pathogenic role of anti-HMGCR in the pathophysiology of this condition, and mimic what we also observed in anti-SRP+ patients.[2]

To conclude, to our knowledge this is the first study outside the United States to confirm the observation and description of anti-HMGCR+ NAM. We showed a marked female predominance, the existence of pediatric cases, and that disease onset may be insidious, leading to an erroneous diagnosis of muscle dystrophy. The majority of our patients were not exposed to statin. The CK level may be a good biomarker for the follow-up of patients who most often need prolonged treatments because of the risk of disease flare, in a disease where anti-HMGCR aAb may have a role in the physiopathology.

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