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ORIGINAL ARTICLE

Clinical spectrums and outcomes of necrotizing autoimmune myopathy versus other idiopathic inflammatory myopathies: a multicenter case-control study

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Abstract

Objective To investigate the clinical characteristics, laboratory features, and treatment outcomes of Thai patients compared between those with necrotizing autoimmune myopathy (NAM) and those with other idiopathic inflammatory myopathies (IIMs) or non-NAM.

Methods This multicenter case-control study included patients aged ≥ 18 years who were diagnosed with IIMs by muscle pathology, and who had relevant clinical and laboratory data, including muscle enzymes, from at least 3 follow-up visits during a 1-year period. Baseline clinical and laboratory data were recorded. Serum myositis-specific autoantibodies (MSAs) were obtained on the date of recruitment.

Results Of the 70 included patients, 67% had NAM, and 33% had non-NAM. The mean age of patients was 50.5 ± 15.9 years, 67% were female, and the median duration of symptoms was 2 months (IQR, 1–4). History of cancer was significantly higher in non-NAM (21.7% vs. 2.1%, $p = 0.01$). Gottron's papules were significantly more prevalent in non-NAM (21.7% vs. 4.3%, $p = 0.04$). Non-NAM had a higher prevalence of anti-Mi-2a (17.4% vs. 2.1%, $p = 0.04$) and Mi-2b (17.4% vs. 0.0%, $p = 0.01$); however, the presence of other MSAs, including anti-HMGCR and anti-SRP, was similar between groups. Improvement in motor power and treatment intensification with glucocorticoid and/or immunosuppressive agents 3 times throughout the follow-up period was similar between groups (NAM 46.8% vs. non-NAM 34.8%, $p = 0.34$).

Conclusion NAM is indistinguishable from non-NAM by clinical manifestations, serology, or laboratory findings, except that pathognomonic skin sign of Gottron's papules and anti-Mi2 are suggestive of dermatomyositis. The integration of clinical, serological, and pathological data is essential for making a diagnosis of NAM.

Key Points
- *NAM is indistinguishable from non-NAM by clinical manifestations, serology, or laboratory findings.*
- *The integration of clinical, serological, and pathological data is essential for making a diagnosis of NAM.*

Keywords Anti-HMGCR · Idiopathic inflammatory myopathy · Myositis-specific autoantibodies · Necrotizing autoimmune myopathy · Outcomes

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Introduction

Idiopathic inflammatory myopathies (IIMs) are a group of autoimmune diseases that cause acute or chronic inflammation of muscles. Common clinical signs and symptoms of IIMs include symmetrical weakness of the proximal muscles of the extremities, acute and insidious onset, elevation of serum muscle enzymes (mostly creatine kinase, [CK]), myopathic pattern abnormalities from electromyography (EMG), and immunopathologic pattern of inflammatory myositis from muscle biopsy, which is the gold standard for confirmation of the diagnosis. Bohan and Peter criteria, which were introduced in 1975, are the most frequently used classification system. These criteria classify IIMs into 5 subgroups, including primary idiopathic polymyositis (PM); primary idiopathic dermatomyositis (DM); DM and PM associated with neoplasia; childhood DM and PM; and DM and PM associated with collagen vascular disease [1, 2]. However, the specificity of this system for the diagnosis of PM is quite low.

During the early 1990s, necrotizing autoimmune myopathy (NAM) was separated from the PM or DM group because it had distinctive histopathological characteristics in the affected muscle. These features include marked muscle necrosis with regeneration in the absence or presence of inflammatory infiltrate, which is different from the other inflammatory myopathies [3]. In 2004, new classification criteria for NAM were proposed at the 119th European Neuromuscular Center (ENMC) workshop that was held in the Netherlands. According to these classification criteria, IIMs are divided into 4 groups, including PM, DM, non-specific myositis, and NAM [4]. When applying the new histopathologic criteria, 20% of IIMs were diagnosed with NAM [5]. Since then, the incidence of NAM may have increased [6].

Serum myositis-specific autoantibodies (MSAs) that are associated with NAM include anti-3-hydroxy-3-methylglutarylcoenzyme-A reductase (anti-HMGCR) and anti-signal recognition particle (anti-SRP). The information related to MSAs utility for the diagnosis and prognosis of NAM is still scarce and controversial.

Regarding treatment response among patients with NAM, 90% required at least 2 immunosuppressive drugs, and 55% frequently relapsed [7].

NAM is a rare IIM. Worldwide, few studies have investigated and reported the clinical characteristics of NAM, including in Thai population. Additionally, anti-HMGCR is still not available in Thailand, so there are still no serological data in Thai population. Accordingly, the aim of this study was to investigate the clinical characteristics, laboratory features, and treatment outcomes of Thai patients compared between those with NAM and those with non-NAM IIMs.

Materials and methods

This multicenter case-control study included eligible patients from the Outpatient Department of three university hospitals located in Bangkok, Thailand (Siriraj Hospital, Ramathibodi Hospital, and Phramongkutklao Hospital). Patients who were at least 18 years of age that were diagnosed with IIMs by muscle biopsy based on ENMC 2004 criteria from the database of the Department of Pathology of each of the 3 participating hospitals, and who had clinical and laboratory data, including muscle enzymes, from at least 3 follow-up visits over a 1-year period during 1 January 2006 to 28 February 2019 were included. Serum samples were obtained from all eligible patients. The protocol for this study was approved by the Institutional Review Board (SIRB) of the Faculty of Medicine Siriraj Hospital, Mahidol University (COA no. SI 040/2018), the Ethical Clearance Committee on Human Rights Related to Research Involving Human Subjects of the Faculty of Medicine Ramathibodi Hospital, Mahidol University (MURA) (COA no. 2018/57), and the Institutional Review Board Committee of the Royal Thai Army Medical Department (IRBRTA) (COA no. S068h/60). All procedures performed in this study that involved human participants were in accordance with the ethical standards of the institutional and/or national research committee, and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standard. Written informed consent was obtained from all individual participants included in this study.

Study population

All eligible subjects were consecutively enrolled and were classified according to the pathological findings of muscle, as follows:

- NAM was defined as many necrotic muscle fibers as the predominant abnormal histologic feature. Inflammatory cells are sparse or only slight perivascular. Perimysial infiltrate is not evident. Major histocompatibility complex class I (MHC class I) antigen, which showed very high sensitivity (98.4%) and low specificity (7.1%) for diagnosis of IIMs and that was upregulated in 88% of patients with anti-HMGCR myopathy [8, 9], was performed in 62% (29/47) of all NAM patients in this study. NAM was divided into 4 groups:

  (1) Idiopathic NAM was defined as NAM with no identified risk factors.
  (2) Statin-associated NAM was defined as receiving statin at symptom onset.

(3) Cancer-associated NAM was defined as cancer identified within 3 years of myopathy onset.
(4) Connective tissue disease (CTD)-associated NAM was defined as CTD known or diagnosed after myopathy onset [7].

- Non-NAM was defined as the other histopathologic criteria for IIMs, including DM or anti-synthetase associated myopathy (ASM), and PM.

– DM or ASM was defined as the presence of perifascicular atrophy (PFA), any of the following perifascicular pathology (perifascular necrosis and regeneration, marked connective tissue [CT] fragmentation), and the presence of mild to moderate CT fragmentation or alkaline phosphatase (ALP) in the perimysium. Additional MxA (Myxovirus resistance A) expression, which had high specificity for DM [10], was performed in only 17% (4/23) of all non-NAM patients.
– PM was defined as endomysial inflammatory cell infiltrate (T cells) surrounding and invading non-necrotic muscle fibers.

Data collection

Muscle pathology

Pathology of the muscle of all eligible patients was independently reviewed by two muscle histopathologists from Siriraj and Ramathibodi Hospitals. Any disagreement was resolved by consensus.

Clinical and laboratory data

Patient characteristics, including demographic data, clinical manifestations, laboratory findings, serology, treatment, and treatment response, were retrospectively collected from medical records from the first diagnosis to the last visit in February 2019.

Initial weakness severity was classified by Medical Research Council (MRC) grade of the weakest muscle group, as follows: none (MRC grade 5/5), mild (MRC grade ≥ 4/5), moderate (MRC grade 3–4/5), and severe (MRC grade < 3/5). Other baseline clinical features at presentation included weakness distribution, constitutional symptoms, and cutaneous features. Cardiovascular involvement was defined by abnormal 12-lead EKG, and interstitial lung disease (ILD) was defined by compatible abnormal chest film and/or computed tomography (CT) of the chest. Laboratory findings consisted of relevant blood panel and muscle enzymes, including creatine kinase (CK), lactate dehydrogenase (LDH), and aspartate aminotransferase (AST) or alanine aminotransferase (ALT). Baseline serology results included any myositis profile without anti-HMGCR, anti-nuclear antibody (ANA; any titers and patterns), and other autoantibody profile.

Treatments and outcomes

Regarding treatment response after 1 year of treatment, we classified motor power improvement into the following 5 levels: no improvement, mild improvement, moderate improvement, marked improvement, and return to baseline [7]. We also classified CK level improvement after treatment into the following 5 levels: no, mild, moderate, good, and excellent improvement based on the ACR/EULAR 2016 criteria [11]. Treatment intensification was defined as the start of or an increase in the dose of corticosteroids by more than 50%, and/or the introduction of immunosuppressive agents.

Myositis profile testing

After obtaining written informed consent, a total volume of 10 ml of blood was obtained. Six milliliters of blood was collected in clotted blood tubes, after which it was spun to separate the 3 ml of serum, and then it was stored at 2–8 °C at the Department of Clinical Pathology for serological testing.

All myositis profiles were performed using the enzyme-linked immunosorbent assay (ELISA) method using the EUROLINE Autoimmune Inflammatory Myopathies Ag (IgG) Test Kit (product no: DL 1530-1601-4G; EUROIMMUN, Lübeck, Germany). This assay consists of the following 15 MSA subsets: anti-Mi-2α, anti-Mi-2β, anti-TIF1-γ, anti-MDA5, anti-NXP2, anti-SAE1, anti-SRP, anti-Jo-1, anti-PL-7, anti-PL-12, anti-EJ, anti-OJ anti-Ku, anti-PM-Scl100, and anti-PM-Scl75. The positive control was included in the test kit, and sample buffer was provided as a negative control. EUROLineScan (EUROIMMUN) was used to detect the signal intensity, and the cutoff threshold was above 10.

The anti-HMGCR autoantibody was performed using the immunoblot method (BlueDot Myositis[7] IgG Immunodot Kit [product no: MYO7D-24]; D-tek, Mons, Belgium]). The positive and negative controls were included in the test kit. The color intensity of the samples was measured by Dr. DOT software and scanning system (D-tek). A level above 10 Dr. DOT arbitrary units (AUs) was regarded as being positive.

Sample size calculation and statistical analysis

To calculate the sample size, we used the following previously reported proportions: the proportion of anti-HMGCR in NAM and in non-NAM IIMs was 31% [12] and 3% [13], respectively. Using a Z of 1.96, a significance level of 5%, and power of 90%, a sample size of 36 patients per group was calculated by nQuery sample size software (Statistical Solutions, Ltd., Cork,

Ireland). To compensate for a potential dropout rate for any reason of 20%, we increased the size of each group by 7 patients. The resulting sample size was 86 patients overall, and 43 patients per group.

Patient characteristics were summarized using descriptive statistics. Categorical data are presented as number and percentage, and continuous data are presented as mean ± standard deviation for normally distributed data, and as median and interquartile range (IQR) for non-normally distributed data. Chi-square test or Fisher's exact test was used to compare categorical variables, and Student's *t* test or Mann-Whitney *U* test was used to compare continuous variables. A *p* value of less than 0.05 was considered statistically significant. All statistical analyses were performed using SPSS Statistics version 20 (SPSS, Inc. Chicago, IL, USA).

Results

Baseline characteristics and demographic data

A total of 70 patients were included in this study (Fig. 1). The mean age of patients was 50.5 ± 15.9 years, 67% were female, and the median duration of symptoms was 2 months (IQR, 1–4). The proportion of patients who had NAM related to statins, cancer, or CTD was less than 25%. History of cancer was significantly higher in non-NAM patients. Based on muscle histopathologic findings, 67.1% were diagnosed with NAM, and 32.9% were diagnosed with non-NAM (Table 1).

Clinical manifestations

Half of patients had moderate proximal muscle weakness of both upper and lower extremities. The most common extramuscular manifestation was unintentional weight loss (60%), followed by various skin signs and ILD. V-sign was the most common skin lesion, followed by shawl sign, heliotrope rash, and nailfold capillary change. When compared between NAM and non-NAM, Gottron's papules were significantly more prevalent in non-NAM. However, other common cutaneous features associated with DM in non-NAM could also be found in patients with NAM (Table 1).

Laboratory and electromyographic findings

The muscle enzyme with the highest level of elevation was CK, with a median level of 25-fold of the upper normal limit. For the other muscle enzymes, the median level of AST, ALT, and LDH was 4.5-fold, 3.1-fold, and 2.5-fold from the upper normal limit, respectively. Electromyography was performed in 70% of patients, and a myopathic pattern was found in 95.9%. Compared between NAM and non-NAM, there was no significantly difference in the levels of all types of muscle enzymes, serum albumin or globulin, ESR, creatinine, or the myopathic patterns from electromyography. Hemoglobin level was significantly lower in non-NAM than in NAM (Table 1).

Serology

Regarding myositis profile at recruitment, anti-Mi-2a and Mi-2b were both significantly more prevalent in non-NAM (Table 2). In contrast, the presence of other autoantibodies at diagnosis, as well as anti-SRP and anti-HMGCR at recruitment, was not significantly different between groups (Table 3). For anti-HMGCR, the prevalence of anti-HMGCR in IIMs was 15.7% (NAM 19% vs. non-NAM 9%, $p = 0.32$). Anti-HMGCR was positive in 67% (6/9) of idiopathic, and in 33% (3/9) of statin-associated NAM. The sensitivity of anti-HMGCR for diagnosis of NAM was 19% (95%

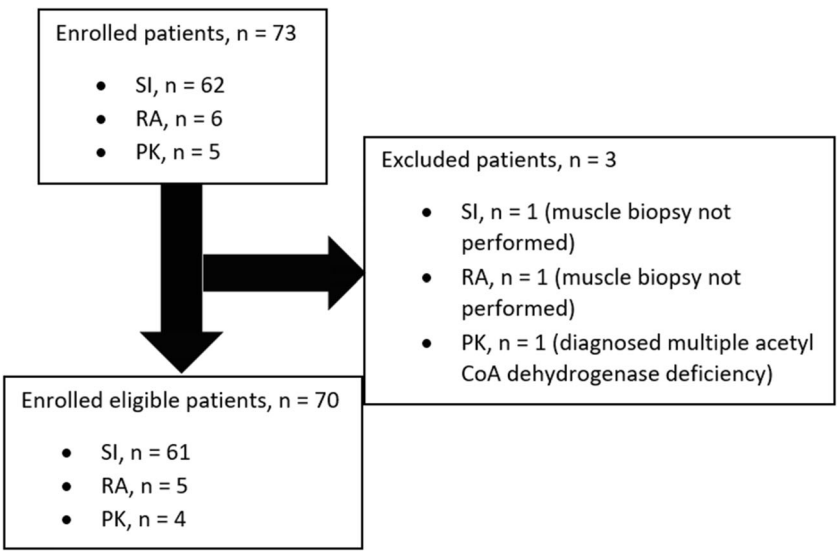

Fig. 1 Study participant disposition. Abbreviations: SI, Siriraj Hospital; RA, Ramathibodi Hospital; PK, Phramongkutklao Hospital

Table 1 Baseline demographic, clinical, laboratory, and electromyographic features of IIM patients

Features	Total ($N = 70$)	NAM ($N = 47$)	Non-NAM ($N = 23$)	P value
Baseline characteristics				
Age at onset (years), mean ± SD	50.5 ± 15.9	52.4 ± 16.0	46.2 ± 15.51	0.11
Female gender, *n* (%)	48 (68.6%)	32 (68.1%)	16 (69.6%)	0.90
Symptom duration (months), median (IQR)	2 (1–4)	2 (1–4)	2 (1–3)	0.47
Prior statins use, *n* (%)	16 (22.9%)	13 (27.7%)	3 (13%)	0.17
Cancer-related, *n* (%)	6 (8.6%)	1 (2.1%)	5 (21.7%)	0.01*
CNTD-related, *n* (%)	12 (17.1%)	7 (14.9%)	5 (21.7%)	0.51
Signs/symptoms				
Myalgia, *n* (%)	38 (54.3%)	28 (59.6%)	10 (43.5%)	0.20
Initial weakness severity, *n* (%)				
• None	4.3% (3/70)	6.4% (3/47)	0% (0/23)	0.57
• Mild	27.1% (19/70)	29.8% (14/47)	21.7% (5/23)	
• Moderate	50% (35/70)	44.7% (21/47)	60.9% (14/23)	
• Severe	18.6% (13/70)	19.1% (9/47)	17.4% (4/23)	
Weakness distribution				
• Symmetric, *n* (%)	65 (92.9%)	43 (91.5%)	22 (95.7%)	1.00
• Proximal, *n* (%)	41 (58.6%)	24 (51.1%)	17 (73.9%)	0.06
• Distal, *n* (%)	18 (25.7%)	10 (21.3%)	8 (34.8%)	0.23
• Dysphagia, *n* (%)	19 (27.1%)	10 (21.3%)	9 (39.1%)	0.12
• Dysarthria, *n* (%)	6 (8.6%)	4 (8.5%)	2 (8.7%)	1.00
Skin manifestations				
• Gottron's papules, *n* (%)	7 (10.0%)	2 (4.3%)	5 (21.7%)	0.04*
• Heliotrope rash, *n* (%)	9 (12.9%)	3 (6.4%)	6 (26.1%)	0.05
• Gottron's sign, *n* (%)	4 (5.7%)	2 (4.3%)	2 (8.7%)	0.59
• V-sign, *n* (%)	10 (14.3%)	4 (8.5%)	6 (26.1%)	0.07
• Shawl sign, *n* (%)	9 (12.9%)	3 (6.4%)	6 (26.1%)	0.05
• Holster sign, *n* (%)	4 (5.7%)	2 (4.3%)	2 (8.7%)	0.59
• Mechanic hand, *n* (%)	6 (8.6%)	4 (8.5%)	2 (8.7%)	1.00
Interstitial lung, *n* (%)	10 (14.3%)	8 (17.0%)	2 (8.7%)	0.48
Laboratory results [% patients performed]				
CK (U/L), median (IQR)	4597 (1753-9163) [100%]	4615 (1980-9140) [100%]	3925 (1310-11,978) [100%]	0.92
LDH (U/L), median (IQR)	1217 (738–1848) [62.9%]	1251 (828–1851) [66%]	931 (691–1827) [56.5%]	0.77
AST (U/L), median (IQR)	179 (99–284) [85.7%]	182 (91–242) [83%]	166 (101–358) [91.3%]	0.65
Electromyography				
Performed, *n* (%)	49/70 (70.0%)	34/47 (72.3%)	15/23 (65.2%)	0.54
Myopathic patterns, *n* (%)	47/49 (95.9%)	32/34 (94.1%)	15/15 (100%)	0.13

Reference range: CK, 0–190 U/L; LDH, 240–480 U/L; AST, 0–40 U/L

CNTD, connective tissue disease; *CK*, creatine kinase; *LDH*, lactate dehydrogenase; *AST*, aspartate aminotransferase;

*P value was less than 0.05

CI, 9–33%), with a specificity of 91% (95% CI, 72–99%) and accuracy of 43% (95% CI, 31–55%). The presence of anti-SRP was observed in 10.6% (5/47) of NAM, and in 8.7% (2/23) of non-NAM. Anti-SRP was identified only in idiopathic (80%, 4/5) and CTD-associated NAM (20%, 1/5).

Treatments and outcomes

Treatment with glucocorticoid, immunosuppressive agents, intravenous immunoglobulin, and biologic agents were similar between groups. No patient underwent plasmapheresis.

Table 2 Frequency of myositis antibodies in IIM patients

Myositis antibodies	Total ($N = 70$)	NAM ($N = 47$)	Non-NAM ($N = 23$)	P value
MAA subtypes, *n* (%)				
Ku	5 (7.1%)	2 (0%)	3 (13%)	0.32
PM-Scl 100	3 (4.3%)	1 (2.1%)	2 (8.7%)	0.25
PM-Scl 75	0 (0%)	0 (0.0%)	0 (0%)	NA
Ro-52	15 (21.4%)	10 (21.3%)	5 (21.7%)	1.00
MSA: anti-ARS, *n* (%)				
Jo-1	6 (8.6%)	2 (4.3%)	4 (17.4%)	0.09
PL-7	2 (2.9%)	2 (4.3%)	0 (0%)	1.00
EJ	0 (0%)	0 (0%)	0 (0%)	NA
OJ	1 (1.4%)	1 (2.1%)	0 (0%)	1.00
MSA: others, *n* (%)				
Mi-2a	5 (7.1%)	1 (2.1%)	4 (17.4%)	0.04*
Mi-2b	4 (5.7%)	0 (0.0%)	4 (17.4%)	0.01*
TIF1g	4 (5.7%)	1 (2.1%)	3 (13.0%)	0.10
MDA5	1 (1.4%)	1 (2.1%)	0 (0.0%)	1.00
NXP2	3 (4.3%)	2 (4.3%)	1 (4.3%)	1.00
SAE1	0 (0%)	0 (0%)	0 (0%)	NA
SAE2	0 (0%)	0 (0%)	0 (0%)	NA
SRP	7 (10%)	5 (10.6%)	2 (8.7%)	1.00
HMGCR	11 (15.7%)	9 (19.1%)	2 (8.7%)	0.32

MAA, myositis associated antibodies; *MSA*, myositis-specific antibodies; *ARS*, anti-aminoacyl-tRNA synthetase
*P value was less than 0.05

There was no significant difference between groups in treatment intensification with glucocorticoids and/or immunosuppressive agents 3 times throughout the follow-up period and median number of treatments in the first year (Table 4). Regarding the response to treatment at 1 year of follow-up (Table 5), motor power returned to normal in 61.7% of NAM patients, and in 56.5% of non-NAM patients. Most of them (94%) had excellent CK improvement at the first year with no difference between groups. Median percentage of CK reduction after first year was 95% vs. 98% for NAM vs. non-NMA. One patient with NAM died at the first year of treatment due to ventilator-associated pneumonia.

Table 3 Frequency of other autoimmune antibodies in IIM patients

Other autoimmune antibodies	N/total (%)	NAM (%)	Non-NAM (%)	P value
ANA	42/65 (64.6%)	29/43 (67.4%)	13/22 (59.1%)	0.72
RNP/Sm	5/25 (20%)	4/16 (25%)	1/9 (11.1%)	0.71
Sm	4/26 (15.4%)	3/15 (20%)	1/11 (9.1%)	0.30
Ro	3/15 (20%)	2/10 (20%)	1/5 (20%)	1.00
La	0/15 (0%)	0/10 (0%)	0/5 (0%)	1.00
Scl70	0/15 (0%)	0/9 (0%)	0/6 (0%)	0.55
PM-Scl	1/6 (16.7%)	0/3 (0.0%)	1/3 (3t3.3%)	0.43
Centromere protein B	0/10 (0%)	0/7 (0%)	0/3 (0%)	1.00
PCNA	0/10 (0%)	0/3 (0%)	0/3 (0%)	0.39
dsDNA	4/27 (14.8%)	3/14 (21.4%)	1/13 (7.7%)	0.05
Nucleosome	1/9 (11.1%)	1/6 (16.7%)	0/3 (0%)	1.00
Histone	0/9 (0%)	0/6 (0%)	0/3 (0%)	1.00
Ribosomal P	1/8 (12.5%)	0/5 (0%)	1/3 (33.3%)	0.47
AMA-M2	0/6 (0%)	0/3 (0%)	0/3 (0%)	0.39

Table 4 Treatments in IIM patients

Treatment	Total ($N = 70$)	NAM ($N = 47$)	Non-NAM ($N = 23$)	P value
IVIg	8 (11.4%)	5 (10.6%)	3 (13.0%)	1.000
IV methylprednisolone	9 (12.8%)	7 (14.9%)	2 (8.7%)	0.708
IV dexamethasone	35 (50%)	24 (51.1%)	11 (47.8%)	1.000
Prednisolone	67 (97.7%)	44 (93.6%)	23 (100.0%)	0.546
Azathioprine	53 (75.7%)	35 (74.5%)	18 (78.3%)	0.728
Methotrexate	30 (42.9%)	20 (42.6%)	10 (43.5%)	0.941
Cyclosporin A	8 (11.4%)	5 (10.6%)	3 (13.0%)	1.000
Mycophenolate mofetil	14 (20.0%)	8 (17.0%)	6(26.1%)	0.525
Cyclophosphamide	16 (22.8%)	11 (23.4%)	5 (21.7%)	0.876
Chlorambucil	8 (11.4%)	5 (10.6%)	3 (13.0%)	1.000
Anti-malarial	62 (88.6%)	39 (83%)	23 (100%)	0.196
Rituximab	1 (1.4%)	1 (2.1%)	0 (0.0%)	1.000
Anti-TNFα	1 (1.4%)	1 (2.1%)	0 (0.0%)	1.000
Treatment intensification ≥ 3 times, n (%)	30 (42.9%)	22 (46.8%)	8 (34.8%)	0.34
Number of treatments in the first year, median (IQR)	6 (8.6%)	3 (3–4)	3 (3–4)	0.77

IVIg, intravenous immunoglobulin; *IV*, intravenous; *anti-TNF*, anti-tumor necrotic factorα

Discussion

IIMs comprise a diverse group of autoimmune disorders that have symmetric proximal muscle weakness as their most common feature. The ENMC 2004 classification criteria for IIMs included NAM based on the results of muscle biopsy, which is currently considered the gold standard for diagnosis [4]. Since the prevalence of these disorders is low, we designed the present study as case-control, multicenter study to ensure a sufficient sample size so that we could investigate the clinical characteristics, laboratory features, and treatment outcomes of Thai patients compared between those with NAM and those with non-NAM IIMs.

The age of onset and duration of symptoms in this study were similar to those reported (mean age 49–64 years and median duration of symptoms 3 months) from other studies [7, 14, 15].

Regarding muscle weakness distribution, distal muscle weakness and dysphagia were found in about 20% of NAM patients. The observed prevalence of dysphagia was consistent with the 27–37% rates reported from other studies [7, 14, 16].

Table 5 Outcomes in IIM patients

Outcomes	Levels	Total ($n = 70$)	NAM ($n = 47$)	Non-NAM ($n = 23$)	P value
Motor power improvement at first year, n (%)	No	7 (10%)	3 (6.4%)	4 (17.4%)	0.64
	Mild	4 (5.7%)	2 (4.3%)	2 (8.7%)	
	Moderate	6 (8.6%)	5 (10.6%)	1 (4.3%)	
	Marked	10 (14.3%)	7 (14.9%)	3 (13%)	
	Return to baseline	42 (60%)	29 (61.7%)	13 (56.5%)	
	Death	1 (1.4%)	1 (2.1%)	0 (0%)	
CK improvement at first year, n (%)	Good	3 (4.3%)	2 (4.3%)	1 (4.3%)	0.50
	Excellent	66 (94.3%)	45 (95.7%)	21 (91.3%)	
%CK reduction at first year, median (IQR)		95.5 (82.3–98.3)	94.9 (80.7–98.0)	97.8 (92.2–98.4)	0.98
CK level after treatment at first year (U/L), median (IQR)		123 (71–298)	142 (67–519)	105 (77–219)	0.38

CK, creatine kinase

Interestingly, Gottron's papule, which is a pathognomonic sign for DM, was found in 2 patients with NAM in our study. It is possible that their muscle pathology was modified by treatments with glucocorticoids and immunosuppressive agents. So, they were misdiagnosed as NAM, instead of DM. Another possibility is that these patients actually had overlapping syndrome of NAM with other connective tissue diseases, such as a patient from India that was initially diagnosed with CTD (SLE)-associated NAM with coexisting Gottron's papules, but muscle biopsy proved the diagnosis to be NAM [17].

Consistent with previous studies that reported that NAM patients had a CK level that was 10 times higher than the upper limit of normal (range, 5000–7000 U/L) [7, 14, 18], we observed the initial CK level of patients with NAM in this study to be increased approximately 25 times higher than normal. Furthermore, we found that the CK level in patients with non-NAM could be as high as the levels in those with NAM. Dalakas et al. reported that muscle enzymes in DM or PM could be elevated up to 50-fold [19]. Another study that used Dalakas et al.'s criteria to diagnose IIMs showed that patients with DM and PM had a mean CK level of 781.8 ± 1812 U/L and 1770 ± 1866 U/L, respectively [20].

Regarding the myositis profile, anti-Mi-2a and anti-Mi-2b were significantly prevalent in non-NAM. This may be explained by the strong association of these antibodies with DM. However, in the Johns Hopkins Myositis Cohort, anti-Mi2 was present in only 13% of patients with DM [21]. When DM was compared to other genetic muscle diseases, anti-Mi2 had a sensitivity of 13% and a specificity of 98% [22]. Concerning anti-HMGCR and anti-SRP, both of which had strong association with NAM in previous studies, we found that the prevalence of anti-HMGCR and anti-SRP in the present study were lower than in previous reports. Additionally, both also had positive results in non-NAM. In previous studies of biopsy-proven NAM patients, the prevalence was 9–62% for anti-HMGCR [7, 12, 13, 15, 18, 23], and 0–18% for anti-SRP [18, 23, 24]. While the prevalence anti-HMGCR of non-NAM patients was 0.5–9% (Table 6) [15, 23]. In 2010, Christopher-Stine et al. reported a novel antibody recognizing 200-kd and 100-kd proteins that was later found to be compatible with anti-HMGCR in 62% (16/26) of biopsy-proven NAM patients, and 63% (10/16) of that group had exposure to statins [23]. In 2015, a study from Japan showed that 31% (8/26) of biopsy-proven NAM patients had anti-HMGCR in their sera, and 38% (3/8) of this group had exposure to statins [12]. Furthermore, some studies reported that anti-HMGCR was still detectable, and that its titer did not normalize despite clinical improvement after treatment [25]. However, anti-HMGCR was also found in other IIMs. A large cohort study conducted in 2016 reported that this autoantibody was detected in 45% of NAM, 4.4% of PM, 1.9% of DM, and 6.7% of juvenile DM [13]. This may reflect differences in the methods used for detection of these antibodies, or differences that exist among different ethnic groups. Nevertheless, our study showed that anti-HMGCR has low accuracy and usefulness for diagnosis of NAM.

Kassardjian et al. reported that most patients with NAM required intensive treatment, such as at least 2 immunosuppressive drugs [7]; however, we found that only less than half of patients with NAM required treatment intensification with glucocorticoids or immunosuppressive drugs more than 3 times. We also found that patients with NAM required more treatment intensification than non-NAM, but the difference between groups was not statistically significant. This may be explained by comparable muscle weakness and level of muscle enzymes between groups at baseline.

Table 6 Anti-HMGCR and anti-SRP in patients with inflammatory myopathy in previous studies

Study	NAM			Non-NAM		
	N	Anti-HMGCR	Anti-SRP	*N*	Anti-HMGCR	Anti-SRP
• Christopher-Stine, et al. 2010 [23]	26	62%$	16%	187	0.5%	
• Ellis E, et al. 2012 [24]	23		0%@			
• Kassardjian CD, et al. 2015 [7]	63	34%*				
• Watanabe Y, et al. 2015 [12]	26	31%#				
• Limaye V, et al. 2015 [15]	23	9%#		175	9%#	
• Musset L, et al. 2016 [13]	69	45%*				
• Watanabe Y, et al. 2016 [18]	387	12%#	18%#			

*ELISA (QUANTA Lite assay [Inova Diagnostics, Inc., San Diego, CA, USA])

ELISA (in house assay)

$ Immunoprecipitation (in house assay)

@ Line Immunoblot assay (Myositis Profile 3 EuroLine; Euroimmun, Lubeck, Germany)

The strength of this study is that we integrated muscle pathology findings in our diagnostic approach as a gold standard for the diagnosis of IIMs. Therefore, the diagnosis of IIMs should be more accurate, as compared to only clinical and serology data that was used for diagnosis in other studies [14, 25, 26]. Moreover, the comprehensive histopathologic review by two independent muscle histopathologists that was used in this study reduced the likelihood of human error. Lastly, this study collected data from 3 major urban medical centers so that we could enroll enough patients with NAM, which is a rare disorder.

This study has some limitations. First, the full panel of immunohistochemistry study was not completely performed in all cases because the muscle biopsy in some cases was performed 10 years earlier. Some patients might have been misclassified as being NAM, especially hereditary myopathy or muscular dystrophy; however, we diagnosed IIMs using patient history and physical examination data from medical records, which were not suggestive of hereditary myopathy or muscular dystrophy to confirm the diagnosis. The chance of misclassifying hereditary myopathy or muscular dystrophy as NAM was quite low. Second, we could not enroll an adequate sample in the non-NAM group because the number of patients with DM in this study was lower than expected. Some patients with DM were diagnosed by clinical manifestations of pathognomonic skin lesion without muscle pathology confirmation. So, this group was not eligible for this study. Third, the retrospective nature of this study rendered it vulnerable to missing or incomplete data, especially data relating to non-myositis autoantibodies at baseline. Some information including MRC grading, treatment regimen, treatment compliance, and non-medical treatment (e.g., muscle rehabilitation) that could have had some impact on treatment outcomes could not be comprehensively evaluated. Fourth, the post-treatment effect may also have played some role. Glucocorticoids and immunosuppressive drugs may interfere with the results of muscle biopsy and serology. However, previous studies showed that glucocorticoid use did not influence the presence or degree of inflammatory infiltrates found in muscle biopsies in DM and PM, with a median time of glucocorticoids use before muscle biopsy of 7–14 days (range, 0–105) and 4–5 days (range, 0–60), respectively [27, 28]. In our study, the median time of glucocorticoids use before muscle biopsy was 3 days (range, 0–7). Additionally, we found seroconversion of two separate myositis autoantibody tests in one patient. This patient was diagnosed with statin-associated autoimmune myopathy and had a positive result for anti-HMGCR at diagnosis from Japan. However, the result of the second anti-HMGCR test in this study that was performed 2 years later was negative. This difference in results may be explained by the different methods used for anti-HMGCR testing (house ELISA vs. Immunodot). Fifth, in general practice, only non-NAM patients who have severe disease or who are refractory to treatment usually undergo muscle biopsy to confirm diagnosis; thus, those patients were considered eligible and enrolled in this study. Consequently, the patients with non-NAM that were enrolled in this study were more severe than the average non-NAM patient. This selection bias may have influenced the observed no difference in response to treatment between groups. Lastly, this study was conducted in 3 university hospitals in Bangkok, Thailand, and all of these centers are tertiary referral centers. As a result, the patients with IIM may have had more severe and aggressive disease than patients in other settings. Therefore, the generalizability of the findings of this study may be limited.

In conclusion, NAM is indistinguishable from non-NAM by clinical manifestations, serology, or laboratory findings, except that pathognomonic skin sign of Gottron's papules and anti-Mi2 are suggestive of dermatomyositis. The integration of clinical, serological, and pathological data is essential for making a diagnosis of NAM.

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Authors' contributions All authors made substantial contributions to the conception, design of the work, the acquisition, analysis, interpretation of data, drafted the work or substantively revised it, and approved the submitted version.

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Compliance with ethical standards

The protocol for this study was approved by the Institutional Review Board (SIRB) of the Faculty of Medicine Siriraj Hospital, Mahidol University (COA no. SI 040/2018), the Ethical Clearance Committee on Human Rights Related to Research Involving Human Subjects of the Faculty of Medicine Ramathibodi Hospital, Mahidol University (MURA) (COA no. 2018/57), and the Institutional Review Board Committee of the Royal Thai Army Medical Department (IRBRTA) (COA no. S068h/60). All procedures performed in this study that involved human participants were in accordance with the ethical standards of the institutional and/or national research committee, and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standard. Written informed consent was obtained from all individual participants included in this study.

Disclosures None.

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