The Journal of Rheumatology 2022;49:922–8
doi:10.3899/jrheum.211234
First Release June 15 2022

The Journal of Rheumatology

Clinical Heterogeneity of Patients With Antinuclear Matrix Protein 2 Antibody–Positive Myositis: A Retrospective Cohort Study in China

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ABSTRACT. ***Objective.*** Heterogeneity exists among patients with myositis who have antinuclear matrix protein 2 (anti-NXP2) antibodies, although they usually present with severe muscle weakness. This study aimed to investigate the differences in phenotypes and prognoses among adult patients with myositis who have anti-NXP2 antibodies.

Methods. Adult patients with myositis who have anti-NXP2 antibodies were enrolled from January 2010 to December 2019. Their clinical features and laboratory data were recorded retrospectively. We followed up on their survival status until June 30, 2020. A hierarchical cluster analysis, Kaplan-Meier curves, and classification and regression trees were used to analyze the data.

Results. A total of 70 adult patients with myositis who have anti-NXP2 antibodies were enrolled. All patients experienced muscle weakness. A total of 11 patients did not present with rashes during disease progression, and 43 patients developed dysphagia. In total, 21 patients had interstitial lung disease (ILD), whereas no patients had rapidly progressive ILD. Hierarchical cluster analysis identified 2 clusters. Patients in cluster 1 were younger at disease onset, had a higher incidence of subcutaneous calcification, and had a lower incidence of V sign and shawl sign. Patients in cluster 2 had a higher frequency of ILD, accompanied by lower levels of lymphocytes and higher levels of serum ferritin. Moreover, patients in cluster 2 had worse prognoses.

Conclusion. Patients with myositis who have anti-NXP2 antibodies may present with different phenotypes that are characterized by unique features and prognoses.

Key Indexing Terms: autoantibodies, antinuclear matrix protein 2 antibody, dermatomyositis, phenotype, prognosis

The antinuclear matrix protein 2 (anti-NXP2) antibody belongs to the spectrum of myositis-specific autoantibodies (MSAs); its positivity percentage varies from 1.6% to 25% in adult patients with idiopathic inflammatory myositis (IIM), based on ethnicity.[1,2] Our previous study indicated that 5.2% of Chinese patients with IIM had anti-NXP2 antibodies.[3] This antibody is recognized as a classification criterion of dermatomyositis (DM); thus, it plays an important role in the diagnosis of myositis.[4,5] Generally, MSAs can help predict and monitor clinical manifestations of DM. As expected, adult patients with anti-NXP2 antibodies usually present with severe muscle weakness and subcutaneous calcification.[6-8] However, heterogeneity in patients with anti-NXP2 antibodies is significant according to clinical practice and the scientific literature. A study performed in Japan pointed out that DM sine dermatitis (DMSD) was significantly associated with the presence of anti-NXP2 antibodies.[9] In addition, anti-NXP2 antibodies were also recognized as cancer-associated autoantibodies, with 7% to 37.5% of patients with DM with anti-NXP2 antibodies developing cancer.[10-12]

DM is a highly complex systemic autoimmune disease; therefore, as a subtype of DM, it is important to clarify the phenotype and prognosis of patients with anti-NXP2 antibodies. This study aimed to analyze this issue to fully understand the characteristics of these patients and to establish disease prognosis early.

METHODS

Study population. Patients with clinically suspected IIM (n = 1215) underwent MSA testing from January 2010 to December 2019 at the Department of Rheumatology, China-Japan Friendship Hospital, Beijing,

This study was supported by the Youth Program of the National Natural Science Foundation of China (8140363), the Capital Health Research and Development of Special Programs (grant no. 2014-4-4062), and the Elite Medical Professionals project of the China-Japan Friendship Hospital (grant no. NO.ZRJY2021-GG14).

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The authors declare no conflicts of interest relevant to this article.

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Accepted for publication April 11, 2022.

China. The sample included 47 juvenile patients with age of onset < 18 years. Anti-NXP2 antibodies were detected in 91 patients. Among them, 16 patients were diagnosed with juvenile DM (JDM), and 5 adult patients were diagnosed with overlap syndrome: 2 with DM and systemic sclerosis, 2 with DM and rheumatoid arthritis, and 1 with DM and Sjögren syndrome. In total, 70 adult patients with anti-NXP2 antibodies were enrolled in our study. The study protocol was approved by the Ethics Committee of China-Japan Friendship Hospital (approval number 2016-117).

Clinical data. Patients' demographic and clinical features, as well as their laboratory data, were gathered through a systemic record review at their first visit to our department.

Clinical manifestations included myalgia, muscle weakness, cutaneous involvement, dysphagia, arthritis, and interstitial lung disease (ILD). Severe muscle weakness was defined as difficulty moving against gravity from within 1 month of the onset of the disease, according to the Medical Research Council 5-point scale. Cutaneous features included classic heliotrope rash, V sign, shawl sign, holster sign, mechanic's hands, Gottron sign and papules, cutaneous ulcer, subcutaneous calcification, and subcutaneous edema. Subcutaneous edema was noted by clinical physicians as any nonpitting swelling of the limbs that met the following criteria: it was new with disease onset and it was not associated with other causes of peripheral edema.

Cancer-associated myositis was defined as cancer occurring within 3 years before or after disease onset.[11]

Laboratory data. Laboratory data consisted of a routine blood test, lymphocyte subsets, serum transaminase (ie, alanine aminotransferase and aspartate aminotransferase), creatine kinase, lactate dehydrogenase, albumin and prealbumin, complement C3 and C4, C-reactive protein, erythrocyte sedimentation rate, serum ferritin, and antinuclear antibody.

Assessment of ILD. All enrolled patients underwent high-resolution computed tomography (HRCT) of the chest, and ILD was diagnosed based on the HRCT image analysis. The subset of patients with rapidly progressive ILD (RP-ILD) were defined as those presenting with progressive dyspnea and a worsening of interstitial changes as detected by HRCT within 1 month of the onset of respiratory symptoms.[13] Patterns of ILD, including usual interstitial pneumonia, nonspecific interstitial pneumonia (NSIP), and organizing pneumonia, were classified using HRCT by 2 experienced radiologists, who were blind to the clinical features of these patients.

Assessment of muscle pathology. A total of 55 patients with anti-NXP2 antibodies had muscle biopsies, including 15 patients who had them performed at other hospitals. We described the pathological characteristics qualitatively based on 4 aspects: muscle fiber, inflammatory, vascular, and connective tissue domains.[14] The description of the muscle fiber domain included perifascicular atrophy, muscle fiber necrosis, regeneration, expression of major histocompatibility complex I, and membrane attack complex (MAC) deposition. The inflammatory domain was assessed by the presence of CD4+ T cells, CD8+ T cells, CD20+ B cells, and CD68+ macrophages. The vascular domain was evaluated by the presence of MAC+ capillaries and vascular occlusion. The connective tissue domain was evaluated by the presence of connective tissue hyperplasia and alkaline phosphatase expression.

Detection of anti-NXP2 antibodies. The anti-NXP2 antibody assay was performed using the EUROLINE Autoimmune Inflammatory Myopathies Ag (IgG) test kit, according to the manufacturer's protocol (order no. DL 1530-1601-4G; EUROIMMUN). The positive control was provided by the test kit and the sample buffer was used as a negative control. EUROBlotOne (EUROIMMUN) was used to detect the signal intensity. The definition of being positive for anti-NXP2 antibodies was a result above the cutoff threshold of 25.

Statistical analysis. SPSS (version 21.0; IBM Corp) was used for most statistical analyses. The Kolmogorov-Smirnov test was used to evaluate the distribution of each continuous parameter. Statistical differences in each group were calculated with *t* tests (normal distribution), Mann-Whitney *U* tests (nonnormal distribution), or chi-square tests. Data were expressed as mean (SD) or median (IQR). A hierarchical cluster analysis was used for classification. Classification and regression trees were used to identify predictors that positioned patients into different clusters; this was done using R software (version 3.6.1; The R Foundation). The variables included in the analysis were sex, age of disease onset, myalgia, severe muscle weakness, cutaneous involvement, dysphagia, arthritis, and ILD. Rashes included heliotrope rash, V sign, shawl sign, holster sign, mechanic's hands, Gottron sign and papules, cutaneous ulcer, subcutaneous calcification, and subcutaneous edema. For the survival analysis, Kaplan-Meier curves were carried out on different clusters. All statistical tests were 2-sided, and significance was set at $P < 0.05$.

RESULTS

General characteristics of patients with anti-NXP2 antibody–positive myositis. Patients with anti-NXP2 antibody–positive myositis (n = 70) were predominantly female (n = 43) and had an average age at disease onset of 40.60 (SD 13.94) years (Supplementary Table 1, available with the online version of this article). No patient had other MSAs. A total of 55 patients, including 9 patients without rashes, underwent muscle biopsies. A total of 59 patients were identified as having DM according to the 2018 European Neuromuscular Centre (ENMC) proposed criteria, and 9 patients were diagnosed with possible DMSD according to the 2004 ENMC criteria.[4,5] Moreover, another 2 patients with positive anti-NXP2 antibodies who showed severe proximal muscle weakness and no rashes were also clinically diagnosed as having DMSD without muscle pathology. It should be noted that the 11 patients without rashes were carefully evaluated, and no cutaneous involvement was found.

All patients had proximal muscle weakness during disease progression, including 23 patients with severe muscle weakness (Supplementary Table 1, available with the online version of this article). A total of 43 patients developed dysphagia There were 9 patients with subcutaneous calcification and 26 patients with subcutaneous edema in our cohort. A total of 21 patients had ILD, whereas no patients in our cohort had RP-ILD. The main pattern of ILD was NSIP (15/21, 71%). In total, 4 patients had cancer, including 2 cases of lung cancer (6 and 13 months after DM, respectively), 1 case of breast cancer (8 months after DM), and 1 case of stomach cancer (3 months before DM).

All the patients in our cohort received glucocorticoids. A total of 57 patients received immunosuppressant, including 6 patients who received biological agents (ie, tocilizumab or rituximab). In total, 20 patients received intravenous Ig (Supplementary Table 1, available with the online version of this article).

We followed up on the survival status of these patients up to June 30, 2020. A total of 15 patients were lost to follow-up and 9 patients died. The survival times, from diagnosis to death, of the patients were in the range of 1 to 63 months. There were 4 clinical factors related to prognosis as determined by univariate analysis: age at disease onset, cancer, and cutaneous ulcer were risk factors for death, and duration from disease onset to diagnosis was a protective factor. However, there was no independent factor as determined by logistic regression analysis (Supplementary Table 2, available with the online version of this article).

Clinical features of patients with anti-NXP2 antibody–positive

myositis stratified into different clusters. As the heterogeneity of patients with anti-NXP2 antibodies is significant in clinical practice, patients in our cohort were stratified into 2 clusters according to Figure 1. Cluster 1 included 37 patients and cluster 2 included 33 patients. The comparisons of patients' clinical features and lab examinations between the clusters are shown in Table 1.

The age at disease onset of patients in cluster 1 was younger than that of patients in cluster 2, whereas the disease duration from clinical onset to diagnosis was comparable. The frequency of severe muscle weakness was not significantly different between the clusters. Subcutaneous calcification tended to occur more frequently in cluster 1 ($P = 0.03$), whereas the proportion of patients with V sign ($P < 0.001$) and shawl sign ($P < 0.001$) seemed higher in cluster 2. Importantly, patients in cluster 2 had ILD more frequently than those in cluster 1 (45% vs 16%; $P = 0.008$). The main subtype of ILD was NSIP in clusters 1 and 2 (87% and 67%, respectively). In addition, cluster 2 (12%) had a greater incidence of cancer than cluster 1 (3%), although there was no significant difference between the 2 clusters.

Regarding laboratory examinations, patients in cluster 2 had significantly higher levels of muscle enzymes and serum ferritin than those in cluster 1. However, the levels of albumin and lymphocytes were lower in cluster 2. We also investigated the muscle pathology characteristics based on 4 aspects in 40 patients who had muscle biopsies performed in our department. There were no significant differences in these variables between the 2 clusters in our cohort (Supplementary Table 3, available with the online version of this article).

Prognoses of patients with anti-NXP2 antibody–positive myositis in different clusters. In cluster 1, there were 2 deaths: 1 patient died of cerebral hernia caused by intracranial infection within 1 month, and the other patient died of disease relapse after 24 months of treatment. In cluster 2, a total of 7 patients died, including 2 deaths from infection, 3 deaths from tumors, and 2 deaths from disease progression. It should be noted that no patients died of ILD in either of the clusters. The survival time curves for the 2 clusters are shown in Figure 2. There was a significant difference between the clusters ($P = 0.007$), and patients in cluster 2 seemed to have an obviously worse prognosis. There was a relatively higher percentage of mortality and shorter survival time in cluster 2, and we found, by logistic regression, that heliotrope rash (odds ratio 0.075, 95% CI 0.010-0.584; $P = 0.01$) was an independent protective factor in these patients. As for patients in cluster 1, there did not exist any risk or protective factors, according to univariate analyses or logistic regression.

Predictors for classifying patients with anti-NXP2 antibody–positive myositis into different clusters. Since there were differences in clinical features and prognoses between cluster 1 and cluster 2, it was necessary to identify early predictors for patients with anti-NXP2 antibody–positive myositis that would position them into 1 cluster or the other. Classification and regression trees showed that age at disease onset was the only predictor, with a correct estimation of 85.71%. Patients with an age of onset of more than 40 years were placed into cluster 2 in our cohort (Figure 3).

Because age at disease onset was an important predictor for patients with anti-NXP2 antibody–positive myositis, and patients in cluster 1 seemed to have a greater frequency of subcutaneous calcification, it was unclear whether the clinical characteristics of patients in cluster 1 and patients with JDM who had anti-NXP2 antibodies were similar or not. Next, we compared the clinical features between patients with JDM who had anti-NXP2 antibodies and patients in cluster 1 or cluster 2, respectively (Supplementary Table 4, available with the online version of this article). We found no differences between patients with JDM who had anti-NXP2 antibodies and patients in cluster 1, except for the age of disease onset in our cohort. Patients in cluster 2 had higher frequencies of V sign, ILD, and subcutaneous edema than those with JDM who had anti-NXP2 antibodies.

DISCUSSION

This was an observational and retrospective study on adult

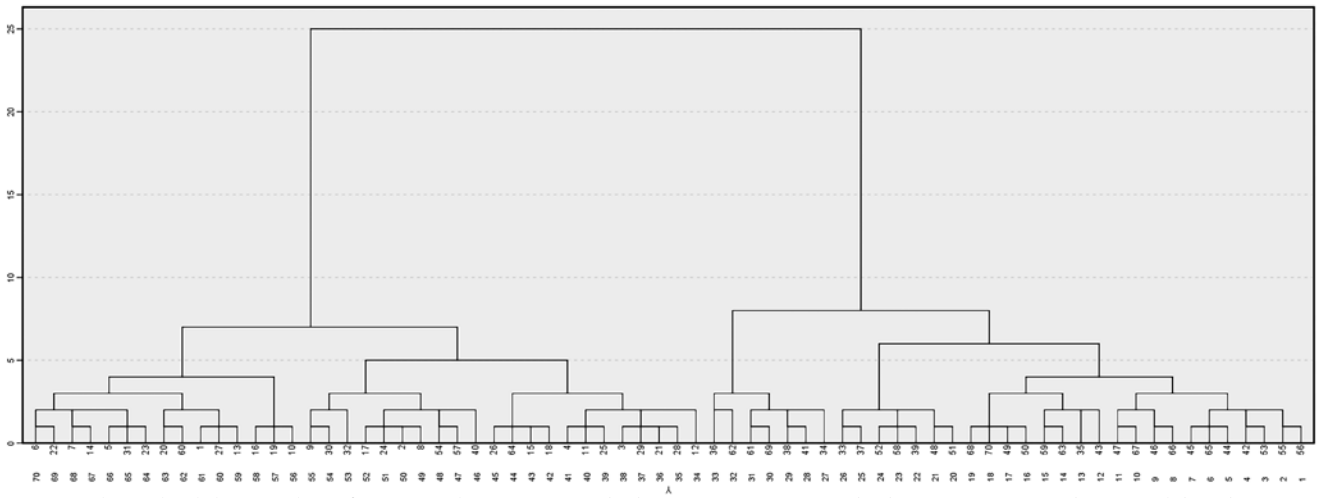

Figure 1. The hierarchical cluster analysis of patients with anti-NXP2 antibody–positive myositis. The dendrogram was generated using Euclidean distance and the Ward agglomerative method. The bold vertical line indicates the height of fusion into the proposed clusters, and the X-axis indicates the individuals (n = 70) at the bottom of the dendrogram. anti-NXP2: antinuclear matrix protein 2.

Table 1. Comparison of clinical features between patients in cluster 1 and cluster 2.

	Cluster 1, n = 37	Cluster 2, n = 33	*P*
Age at onset, yrs, mean (SD)	30.41 (7.83)	52.03 (9.76)	< 0.001**
Age at onset, yrs			
18-30	20 (54)	0 (0)	< 0.001**
31-40	14 (38)	3 (9)	0.005**
41-50	3 (8)	11 (33)	0.008
51-60	0 (0)	13 (39)	< 0.001**
61-70	0 (0)	4 (12)	0.045
> 70	0 (0)	2 (6)	0.22
Sex			0.40
Male	16 (43)	11 (33)	
Female	21 (57)	22 (67)	
Duration from onset to diagnosis, months, median (IQR)	2.0 (1.5-5.0)	2.0 (1.0-3.5)	0.16
Muscle weakness			0.27
Any muscle weakness	37 (100)	33 (100)	
Severe muscle weakness	10 (27)	13 (39)	
Myalgia	29 (78)	25 (76)	0.79
Rash			
All rash types	29 (78)	30 (91)	0.15
Heliotrope rash	23 (62)	22 (67)	0.70
V sign	9 (24)	22 (67)	< 0.001**
Shawl sign	4 (11)	17 (52)	< 0.001**
Holster sign	4 (11)	4 (11)	1.000
Mechanic's hands	3 (8)	7 (19)	0.22
Gottron sign and papules	8 (22)	12 (32)	0.17
Cutaneous ulcer	3 (8)	6 (16)	0.29
Subcutaneous calcification	8 (22)	1 (3)	0.03*
Subcutaneous edema	12 (33)	14 (42)	0.39
Arthritis	2 (5)	3 (9)	0.66
Interstitial lung disease	6 (16)	15 (45)	0.008**
Dysphagia	23 (62)	20 (61)	0.89
WBCs, mean (SD)[a]			
All WBCs, × 10^9/L	8.12 (3.91)	9.23 (4.33)	0.26
Neutrophils, × 10^9/L	6.24 (3.81)	7.61 (4.06)	0.15
Lymphocytes			
All lymphocytes, × 10^9/L	1.23 (0.67)	0.97 (0.36)	0.04*
CD3, cells/μL	850.65 (515.96)	741.35 (365.80)	0.33
CD4, cells/μL	557.29 (344.89)	511.52 (280.31)	0.56
CD8, cells/μL	273.85 (213.90)	212.77 (113.26)	0.15
NK, cells/μL, median (IQR)	31.00 (19.50-64.25)	46.50 (28.25-80.75)	0.08
B1 (cells/μL)	63.65 (74.71)	46.97 (64.53)	0.35
B2 (cells/μL)	248.85 (180.29)	244.76 (32.65)	0.94
ALT, U/L, median (IQR)	48.0 (25.5-92.0)	67.0 (38.0-157.5)	0.06
AST, U/L, median (IQR)	45.0 (22.0-107.5)	132.0 (38.5-271.0)	0.02*
CK, U/L, median (IQR)	233.0 (95.5-1685.0)	1462.0 (177.0-6163.0)	0.01*
LDH, U/L, mean (SD)	383.65 (238.32)	590.73 (370.32)	0.006**
Albumin, g/L, mean (SD)	36.69 (5.29)	33.19 (7.23)	0.02*
Prealbumin, mg/L, mean (SD)	220.10 (98.05)	182.72 (74.67)	0.08
C3, mg/dL, mean (SD)	75.24 (25.79)	86.32 (17.93)	0.04*
C4, mg/dL, mean (SD)	16.94 (6.43)	20.16 (4.95)	0.02*
CRP, mg/dL, median (IQR)	0.44 (0.19-0.94)	0.63 (0.39-1.27)	0.12
ESR, mm/h, median (IQR)	11.0 (7.0-21.5)	16.0 (8.0-33.0)	0.16
Serum ferritin, ng/mL, median (IQR)	139.2 (54.0-606.0)	312.9 (145.4-768.5)	0.01*
ANA positive	13 (35)	17 (52)	0.17
Cancer-associated myositis	1 (3)	3 (12)	0.34[b]
Muscle biopsy	30 (81)	25 (76)	0.59

Data are in n (%) unless otherwise indicated. [a] All WBC values are reported as mean (SD), except for NK, which is reported as median (IQR). [b] These data were deleted because patients were lost to follow-up. * $P < 0.05$. ** $P < 0.01$. ALT: alanine aminotransferase; ANA: antinuclear antibody; AST: aspartate aminotransferase; CK: creatine kinase; CRP: C-reactive protein; ESR: erythrocyte sedimentation rate; LDH: lactate dehydrogenase; NK: natural killer; WBC: white blood cell.

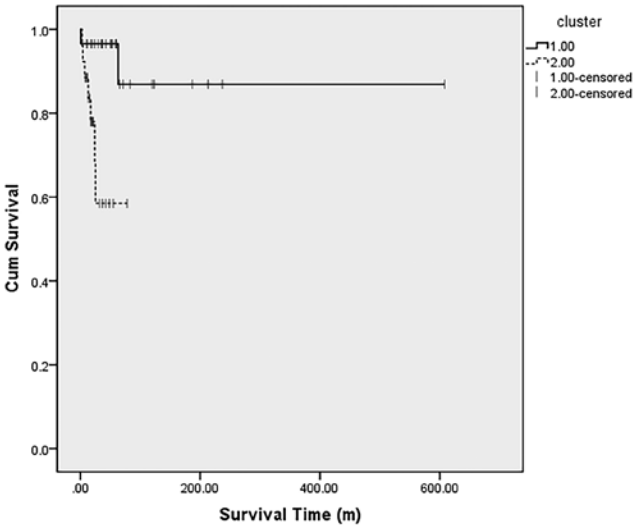

Figure 2. The comparison of survival time between cluster 1 and cluster 2. Cum: cumulative; m: months.

patients with anti-NXP2 antibody–positive myositis in China. We identified 2 clusters of patients by hierarchical cluster analysis. According to our exploratory study results, patients in cluster 1 had a lower age of disease onset and a greater frequency of subcutaneous calcification. Further, their clinical characteristics were somewhat similar to those of patients with JDM. Patients in cluster 2 had a greater incidence of ILD and worse prognoses. This indicated that more attention should be paid to patients with anti-NXP2 antibody–positive myositis with a higher age at disease onset because of their poor outcomes.

Muscle weakness and rashes were the characteristic features of patients with DM who had anti-NXP2 antibodies, especially regarding severe muscle weakness and subcutaneous calcification.[7,15] Previous studies suggested that patients with anti-NXP2 antibodies might exhibit three main DM subtypes: classic DM, amyopathic DM (ADM), and DMSD.[4,5,9,16] ADM and DMSD were significantly associated with anti–melanoma differentiation–associated gene 5 (anti-MDA5) and anti-NXP2 antibodies in clinical practice, respectively.[9,17] In our study, some patients had possible DMSD according to the 2004 ENMC criteria; however, these cases of DMSD could not be classified as DM according to the 2018 ENMC proposed criteria because they did not have the typical rashes found in DM. As we know, subcutaneous calcification correlated with the presence of this antibody, both in patients with JDM and adult patients with DM.[18,19] However, we found that only young adult patients, especially those younger than 40 years of age in our cohort, had a higher risk of developing subcutaneous calcification. This risk was lower in older patients, which meant that adult patients with a higher age at disease onset had a lower frequency of subcutaneous calcification. Further, 1 study indicated that calcinosis was rare among Chinese adult patients with anti-NXP2 antibody–positive myositis.[20] Therefore, in addition to the presence of anti-NXP2 antibodies, subcutaneous calcification might also be associated with both age and ethnicity in adults. Generally, muscle weakness is severe in patients with anti-NXP2

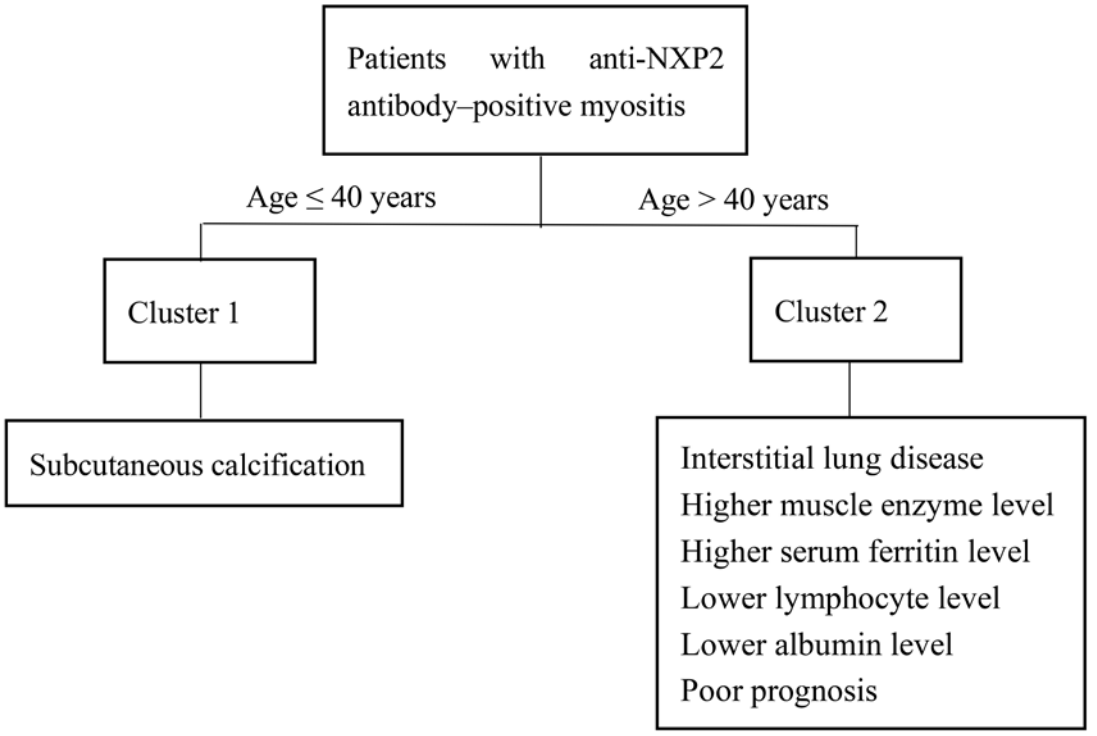

Figure 3. The classification and regression trees of each cluster. The regression tree model was used to identify the class within which a target variable would presumably fall. Using classification and regression trees, age of disease onset was identified as a predictor that positioned patients into different clusters. The main clinical characteristics of the different clusters are shown in the lower boxes. anti-NXP2: antinuclear matrix protein 2.

antibodies.[7,16] In our study, more than 30% of patients had severe muscle weakness. Older patients normally had higher muscle enzyme levels, whereas the proportions of severe muscle weakness in the 2 clusters were comparable.

The incidence of ILD in patients with anti-NXP2 antibody–positive DM varied greatly among previous studies.[2,3,21-23] In our cohort, ILD was not rare, with a prevalence of 30% in patients with anti-NXP2 antibody–positive myositis. The main subtype of ILD was NSIP, which is consistent with what has been observed for patients with IIM.[24,25] However, it should be noted that ILD in these patients was not serious. No patients had RP-ILD, and no death from infection was observed in patients with ILD. In our study, almost half of the patients in cluster 2 suffered from ILD. They had lower levels of lymphocytes and higher serum ferritin levels, which were considered to be indicators of poor prognosis in anti-MDA5 antibody–positive DM.[26,27]

In our study, 9 patients died because of poor control of disease progression, severe infections, and tumors. Patients with anti-NXP2 antibody–positive myositis usually develop severe muscle weakness, including pharyngeal and respiratory muscles.[7] This rapid progression of muscle weakness may induce suffocation by bucking and dyspnea. Pneumonia followed aspiration caused by dysphagia. Timely supporting treatment was necessary for these patients. In addition, the anti-NXP2 antibody was found to be a cancer-associated antibody, and the incidence rate of cancer increased with age.[12,28] Patients in cluster 2 who were older appeared to have worse prognoses and survival times; the reasons were complex. They seemed to have more internal organ involvement. In addition, the higher incidence of cancer might have reduced the survival rates. These data indicate that greater attention should be paid to patients who are older at disease onset, regardless of the supporting treatment and cancer screening results.

This study had several obvious limitations. First, it had a retrospective and observational design, and all data were based on previous medical records. We focused only on the clinical heterogeneity of patients who were positive for anti-NXP2 antibodies, and we did not analyze the clinical differences between patients with different titers of anti-NXP2 antibodies. As for the muscle biopsy, we only described the muscle pathology qualitatively instead of applying dedicated scores to obtain more accurate results. Second, all patients were from a single center, which hampered the avoidance of possible biases and led to a restricted number of cases. Some of the data were censored, which might have affected the statistical analysis of prognosis. Therefore, larger cohorts from multiple centers are necessary for further investigation in the future. In addition, the anti-NXP2 antibodies were measured using EUROLINE test kits, which can generate false positive or negative results. However, the use of EUROLINE test kits is a commonly used method in clinical practice at present. Although different studies may have inconsistent results, a previous study showed that there was good agreement when test kit results were compared to the gold standard of immunoprecipitation.[29] In addition, it is worth noting that all the statistical analyses were applied to a small sample cohort, which might have led to potential limitations in the results. Therefore, a larger sample cohort is needed for validation.

In conclusion, we detected clinical and prognostic heterogeneity in patients with anti-NXP2 antibody–positive myositis. Two clusters of patients were identified in this exploratory study. Patients in 1 cluster were younger at disease onset and had a higher frequency of subcutaneous calcification. Patients in the other cluster seemingly had more internal organ involvement and worse prognoses.

ONLINE SUPPLEMENT

Supplementary material accompanies the online version of this article.

REFERENCES

1. Stuhlmüller B, Schneider U, González-González JB, Feist E. Disease specific autoantibodies in idiopathic inflammatory myopathies. Front Neurol 2019;10:438.
2. Li L, Wang H, Wang Q, et al. Myositis-specific autoantibodies in dermatomyositis/polymyositis with interstitial lung disease. J Neurol Sci 2019;397:123-8.
3. Li S, Ge Y, Yang H, et al. The spectrum and clinical significance of myositis-specific autoantibodies in Chinese patients with idiopathic inflammatory myopathies. Clin Rheumatol 2019;38:2171-9.
4. Hoogendijk JE, Amato AA, Lecky BR, et al. 119th ENMC international workshop: trial design in adult idiopathic inflammatory myopathies, with the exception of inclusion body myositis, 10-12 October 2003, Naarden, the Netherlands. Neuromuscul Disord 2004;14:337-45.
5. Mammen AL, Allenbach Y, Stenzel W, Benveniste O, ENMC 239th Workshop Study Group. 239th ENMC international workshop: classification of dermatomyositis, Amsterdam, the Netherlands, 14-16 December 2018. Neuromuscul Disord 2020;30:70-92.
6. DeWane ME, Waldman R, Lu J. Dermatomyositis: clinical features and pathogenesis. J Am Acad Dermatol 2020;82:267-81.
7. Albayda J, Pinal-Fernandez I, Huang W, et al. Antinuclear matrix protein 2 autoantibodies and edema, muscle disease, and malignancy risk in dermatomyositis patients. Arthritis Care Res 2017;69:1771-6.
8. Satoh M, Tanaka S, Ceribelli A, Calise SJ, Chan EKL. A comprehensive overview on myositis-specific antibodies: new and old biomarkers in idiopathic inflammatory myopathy. Clin Rev Allergy Immunol 2017;52:1-19.
9. Inoue M, Tanboon J, Hirakawa S, et al. Association of dermatomyositis sine dermatitis and with anti-nuclear matrix protein 2 autoantibodies. JAMA Neurol 2020;77:872-7.
10. Ichimura Y, Matsushita T, Hamaguchi Y, et al. Anti-NXP2 autoantibodies in adult patients with idiopathic inflammatory myopathies: possible association with malignancy. Ann Rheum Dis 2012;71:710-3.
11. Yang H, Peng Q, Yin L, et al. Identification of multiple cancer-associated myositis-specific autoantibodies in idiopathic inflammatory myopathies: a large longitudinal cohort study. Arthritis Res Ther 2017;19:259.
12. Lu X, Peng Q, Wang G. The role of cancer-associated autoantibodies as biomarkers in paraneoplastic myositis syndrome. Curr Opin Rheumatol 2019;31:643-9.
13. Lian X, Zou J, Guo Q, et al. Mortality risk prediction in amyopathic dermatomyositis associated with interstitial lung disease: the FLAIR model. Chest 2020;158:1535-45.
14. De Bleecker JL, De Paepe B, Aronica E, et al. 205th ENMC international workshop: pathology diagnosis of idiopathic inflammatory myopathies Part II, 28-30 March 2014, Naarden, The Netherlands. Neuromuscul Disord 2015;25:268-72.

15. Betteridge Z, Tansley S, Shaddick G, et al. Frequency, mutual exclusivity and clinical associations of myositis autoantibodies in a combined European cohort of idiopathic inflammatory myopathy patients. J Autoimmun 2019;101:48-55.
16. Liu Y, Zheng Y, Gang Q, et al. Perimysial microarteriopathy in dermatomyositis with anti-nuclear matrix protein-2 antibodies. Eur J Neurol 2020;27:514-21.
17. Moghadam-Kia S, Oddis CV, Aggarwal R. Anti-MDA5 antibody spectrum in Western world. Curr Rheumatol Rep 2018;20:78.
18. Zhong L, Yu Z, Song H. Association of anti-nuclear matrix protein 2 antibody with complications in patients with idiopathic inflammatory myopathies: a meta-analysis of 20 cohorts. Clin Immunol 2019;198:11-8.
19. Chung MP, Richardson C, Kirakossian D, et al. Calcinosis biomarkers in adult and juvenile dermatomyositis. Autoimmun Rev 2020;19:102533.
20. Wang L, Huang L, Yang Y, et al. Calcinosis and malignancy are rare in Chinese adult patients with myositis and nuclear matrix protein 2 antibodies identified by an unlabeled immunoprecipitation assay. Clin Rheumatol 2018;37:2731-9.
21. Bermudez J, Heim X, Bertin D, et al. Lung involvement associated with anti-NXP2 autoantibodies in inflammatory myopathies: a French monocenter series. Expert Rev Respir Med 2020;14:845-50.
22. Rogers A, Chung L, Li S, Casciola-Rosen L, Fiorentino DF. Cutaneous and systemic findings associated with nuclear matrix protein 2 antibodies in adult dermatomyositis patients. Arthritis Care Res 2017;69:1909-14.
23. Long K, Danoff SK. Interstitial lung disease in polymyositis and dermatomyositis. Clin Chest Med 2019;40:561-72.
24. Ahuja J, Arora D, Kanne JP, Henry TS, Godwin JD. Imaging of pulmonary manifestations of connective tissue diseases. Radiol Clin North Am 2016;54:1015-31.
25. Zuo Y, Ye L, Liu M, et al. Clinical significance of radiological patterns of HRCT and their association with macrophage activation in dermatomyositis. Rheumatology 2020;59:2829-37.
26. Motegi SI, Sekiguchi A, Toki S, et al. Clinical features and poor prognostic factors of anti-melanoma differentiation-associated gene 5 antibody-positive dermatomyositis with rapid progressive interstitial lung disease. Eur J Dermatol 2019;29:511-7.
27. Romero-Bueno F, Diaz Del Campo P, Trallero-Araguás E, et al. Recommendations for the treatment of anti-melanoma differentiation-associated gene 5-positive dermatomyositis-associated rapidly progressive interstitial lung disease. Semin Arthritis Rheum 2020;50:776-90.
28. Fiorentino DF, Chung LS, Christopher-Stine L, et al. Most patients with cancer-associated dermatomyositis have antibodies to nuclear matrix protein NXP-2 or transcription intermediary factor 1γ. Arthritis Rheum 2013;65:2954-62.
29. Cavazzana I, Fredi M, Ceribelli A, et al. Testing for myositis specific autoantibodies: comparison between line blot and immunoprecipitation assays in 57 myositis sera. J Immunol Methods 2016;433:1-5.