Identification of 4 subgroups of juvenile dermatomyositis by principal component analysis-based cluster analysis

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Abstract

Objective

Juvenile dermatomyositis (JDM) is an autoimmune disease characterised by a great heterogeneity in its clinical manifestations. In this study, we aimed to investigate the association between different clinical subtypes, laboratory data, and myositis antibodies of JDM.

Methods

A total of 132 JDM patients were enrolled and their medical records were retrospectively reviewed and autoantibodies tested. Twenty-one variables, including clinical manifestations and laboratory findings, were selected for analysis. We selected principal component analysis (PCA) as a pre-processing method for cluster analysis to convert the 21 original variables into independent principal components. We then conducted a PCA-based cluster analysis in order to analyse the association between patient clusters and the clinical data, laboratory data, and myositis autoantibodies.

Results

We identified 4 distinct JDM subgroups by PCA-based cluster analysis, namely: cluster A, JDM patients with arthralgia and intense inflammation; cluster B, JDM patients with clinical manifestations of vasculitis; cluster C, hypermyopathic JDM patients; and cluster D, JDM patients with skin involvement. There were significant differences between the 4 groups in serum alkaline phosphatase levels, usage of aggressive immunosuppressive therapy, and autoantibody expression of anti-mi2, anti-MDA5, anti-Jo1, and anti-PM-Scl100.

Conclusion

We conducted cluster analysis of a cohort of JDM patients and identified 4 subgroups that represented diverse characteristics in the distribution of laboratory data and myositis autoantibodies, indicating that multidimensional assessment of clinical manifestations is highly valuable and urgently needed in JDM patients. These subgroups may contribute to individualised treatments and improved JDM patient prognosis.

Key words

principal component analysis, cluster analysis, juvenile dermatomyositis, autoantibody

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Introduction

As one of the most prevalent inflammatory myopathies in children, juvenile dermatomyositis (JDM) affects 1.9 patients per million children in the United Kingdom (1) and 2.4–4.1 patients per million children in the USA (2). The mortality rate of JDM in developed countries is currently estimated at 2–3% (3). JDM has been proven to be of great heterogeneity: the clinical symptoms are widely diverse and include muscle and skin involvement, interstitial lung disease (ILD), arthritis, and cardiac damage.

Of note, JDM shares criteria with DM. The identification of clinical DM phenotypes tends to be of great significance for the prognosis and has been the focus of extensive research. Bohan and Peter (4, 5) reported 4 subtypes of DM, and juvenile DM is one of them; the other three subtypes are DM associated with malignancy, DM associated with other connective tissue diseases (CTD), and idiopathic DM. The European League Against Rheumatism (EULAR)/American College of Rheumatology (ACR) (6) have validated classification criteria for adult and juvenile idiopathic inflammatory myopathies (IIM) and their major subgroups. However, studies dedicated to identifying the subtypes of DM, and especially of JDM, are still scarce.

We designed this study to investigate the association between different clinical subtypes, laboratory data, and myositis antibodies in JDM. In this study, we adopted a principal component analysis (PCA)-based cluster analysis as an exploratory method, which proved to be suitable for identifying JDM subtypes. Four distinct JDM subtypes were identified and then validated by detecting significant differences in immunesuppressive therapies, laboratory data, and expression of myositis antibodies.

Method

Population

A total of 132 patients diagnosed with JDM from inpatient wards of the Beijing Children's Hospital between June 2015 and September 2018 were enrolled in our study. The Bohan and Peter criteria were applied to diagnose JDM (4, 5). Patients with juvenile polymyositis (JPM) were excluded to reduce the interference, as JPM has already been identified as a distinct subtype with a different pathological mechanism compared with JDM. Other myopathies were also excluded. Ethics approval and informed patient consent were obtained from patients and their guardians.

Data extraction

Data at the time of first hospitalisation were collected from the patients' medical charts. These included the following parameters: demographics, IIM-related clinical manifestations, laboratory findings, and immunosuppressive therapy. We also documented the administration of aggressive immunosuppressive therapy, which was defined as a repeated corticosteroid impulse therapy for ≥2 times. We chose methylprednisolone (MP) for impulse therapy with a dose of 10–20 mg/kg/d, 3–5 days per pulse.

Data analysis

In recent years, grouping techniques have become prominent analysis methods. As one of the most popular methods of unsupervised learning, cluster analysis has shown great advantage in identifying subgroups by similar characteristics (7). In this study, cluster analysis was performed to achieve subtyping in JDM patients. Four critical steps were performed in the statistical analysis, including selection of clinical variables, cluster analysis of these variables to explore the relationships between them, PCA to reduce interactions between the variables, and cluster analysis of patients based on the PCA-transformed data.

Variables with abnormal distribution were expressed as median and then compared using non-parametric tests. Categorical data are presented as numbers (percentages), and the chi-square test was utilised.

Variable selection

We selected 21 variables frequently found in the JDM and detected myositis antibodies and then included these original variables in the analysis (Table I). The 21 variables were included in the

Table I. Clinical characteristics and antibody positive rate of 132 patients with juvenile dermatomyositis.

	Patients, n (%) (n=132)
Demographics	
Female	67 (50.8)
Age at onset[b], years	8.1 (3.7)
Course of disease[a], months	10.0 (20.7)
Clinical features[a]	
Heliotrope rash	98 (74.2)
Gottron sign	102 (77.3)
Muscle weakness	109 (82.6)
Myalgia/muscle tenderness	21 (15.9)
Eyelid swelling	21 (15.9)
Calcinosis cutis	20 (15.2)
Digital ulcer	7 (5.3)
Fever	46 (34.8)
Raynaud's phenomenon	3 (2.3)
Cough	33 (25.0)
Periungual telangiectasia	26 (19.7)
Arthritis/arthralgia	33 (25.0)
Dysphagia	12 (9.1)
Choking cough	15 (11.4)
Hoarseness	6 (4.5)
Alopecia	4 (3.0)
WBC[c]	8.4 (3.6)
Fatigue	109 (82.6)
Movement limitation	5 (3.8)
Chiblian rash	2 (1.5)
Laboratory data	
Creatine kinase level[b]U/L	163.0 (119.0)
ALP level[c],U/L	144.1 (84.0)
Repeated corticosteroid impulse therapy	73 (55.3)
Antibody	
Mi2	18 ()
TIFγ	26 (19.7)
MDA5	16 (12.1)
NPX2	30 (22.7)
SAE1	1 (0.8)
Ku	8 (6.1)
PMScl100	7 (5.3)
PMScl75	9 (16.8)
Jo1	3 (2.3)
SRP	8 (6.1)
PL7	3 (2.3)
PL12	3 (2.3)
EJ	0 (0.0)
OJ	5 (3.8)
RO52	44 (33.3)

[a]Variables used for the creation of clusters. [b]Values are expressed as medians (interquartile ranges). [c]Values are expressed as the mean (standard deviation).

analysis, with the following details: 1. frequently found and available in JDM; 2. variables with no large-scale missing data; and 3. variables with similar significance, such as myalgia and muscle tenderness, were combined into a novel variable for further analysis. Each of these variables represented heterogeneous or homologous pathogenesis, which can be verified by clustering. Western blotting and 16 items of the anti-myositis spectrum kit (European) were used to detect serum antibodies in 132 patients, including anti-Mi-2α, anti-Mi-2 β, anti-TIF1-γ, anti-MDA5, anti-NXP2, anti-SAE1, anti-Ku, anti-PM-Scl100, anti-PM-Scl75, anti-Jo-1, anti-SRP, anti-PL-7, anti-PL-12, anti-EJ, anti-OJ, anti-Ro-52. Continuous variables, such as age at onset and CK level, were standardised by evaluating the distribution, mean value, median value, and extremum of the continuous variables. Then, the variables were standardised by removing the mean and scaling unit variance.

Identification of distinct clusters

We performed categorical PCA (CATPCA) to detect critical features and reduce dimensionality of the original variables, for which our data was mixed with both continuous and binary variables. By reducing variances or eigenvalues, the original variables were transformed into 21 independent components for cluster analysis. Agglomerative clustering algorithms were applied to cluster variables. Hierarchical clustering hierarchically combined clusters with the smallest distances (7). Through this process, the Ward method was selected to decrease the total within-cluster variance; then, squared Euclidean distance was applied for similarity measurement. For continuous normally distributed variables, differences between the clusters were compared using non-parametric tests including the Kruskal-Wallis test, and the chi-square test or Fisher's exact test was used for categorical variables. $p<0.05$ was considered statistically significant.

Results

Characteristics of variables

Among the 132 JDM patients enrolled in our study, 67 (50.8%) were female (Table I). The median age at onset was 8.1 years (2.0–15.5 years), and the median duration of the disease was 10.0 months (0.5–60.0 months). Muscle weakness (82.6%) and fatigue (82.6%) were the most prevalent clinical manifestations, followed by Gottron sign (77.3%), heliotrope rash (74.2%), fever (34.8%) and arthritis/arthralgia (25.0%). The median CK level was 163.0 U/L (11.0–18397.0 U/L), and alkaline phosphatase (ALP) level was 144.1±84.0 U/L. A total of 75 (50.3%) patients received repeated corticosteroid impulse therapy.

CATPCA

Twenty-one independent principal components were obtained by CATPCA of the original variables. These principal components explained all the variance and were included in the cluster analysis.

Cluster analysis

Hierarchical cluster analysis was conducted on the 132 patients on the basis of CATPCA. Figure 1 displays the results of the hierarchical cluster analysis of the 21 clinical variables. Figure 2 shows the process of clustering of the patients. On the basis of the equipartition principle, the clustering resulted in 4 clusters.

Relationship between ALP, immunosuppressive therapy, antibodies and clusters

To validate the classification, we examined the relationship between the clusters and laboratory data, immunosuppressive therapy, and expression of antibodies. These parameters were not used for clusters. The results of this analysis are presented in Table II. There were significant differences between the 4 groups in serum ALP levels, usage of aggressive immunosuppressive therapy, and autoantibody expression of anti-mi2, anti-MDA5, anti-Jo1, and anti-PM-Scl100.

Discussion

We conducted a PCA-based cluster analysis to analyse the clinical data, laboratory data, and myositis autoantibodies of a cohort of patients with JDM. The study identified 4 subgroups according to the clinical data: cluster A, JDM patients with arthralgia and intense inflammation; cluster B, JDM patients with clinical manifestations of vasculitis; cluster C, hypermyopathic JDM patients; cluster D, JDM patients with skin involvement. These 4 subgroups represented diverse characteristics in the dis-

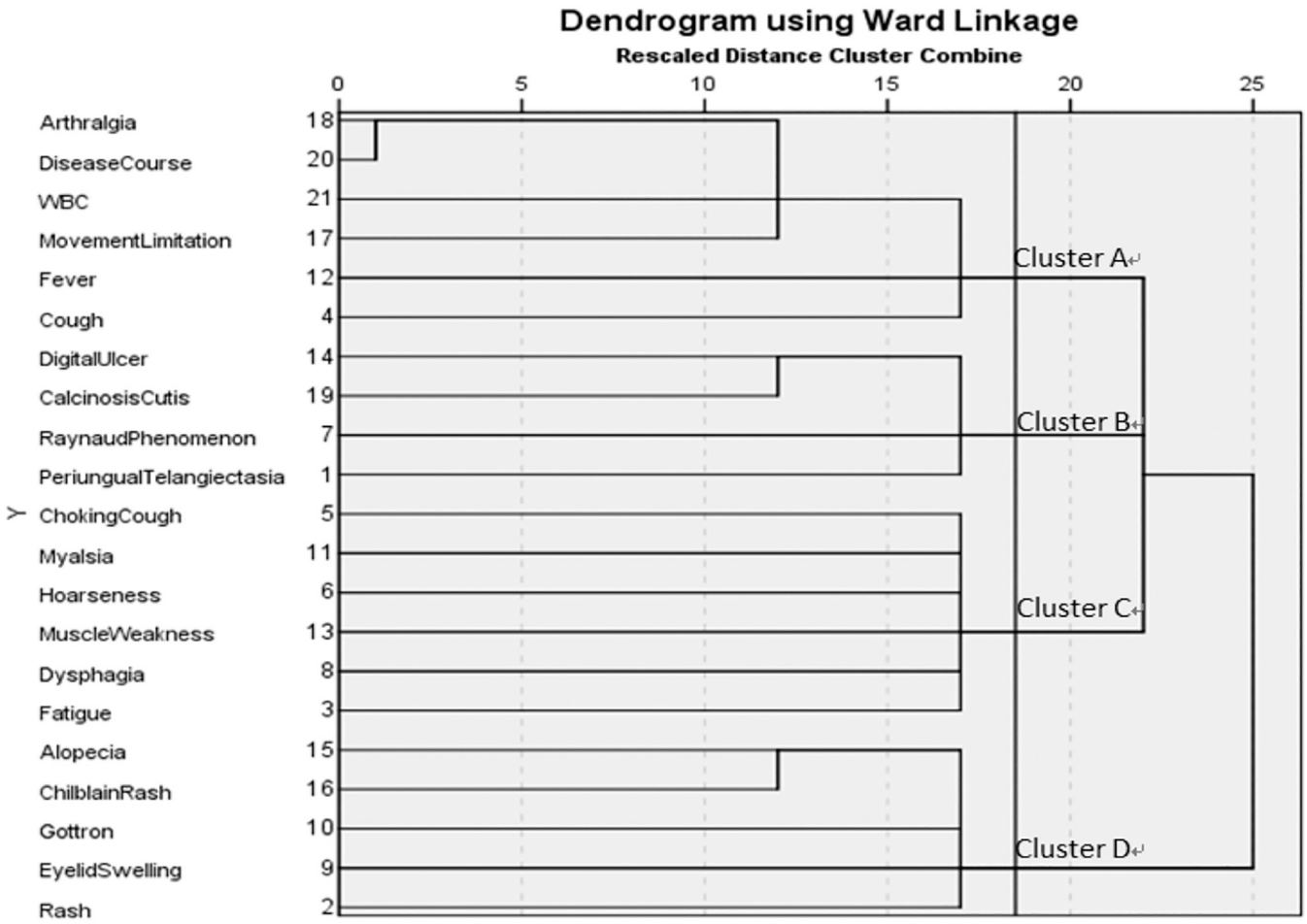

Fig. 1. Dendrogram showing the process and results of hierarchical cluster analysis of 21 variables.
The horizontal axis represents the rescaled distance cluster combination in which the largest distance between clusters was marked as 25. Horizontal lines on the left represent the clustering observations, which in this case are clinical variables.

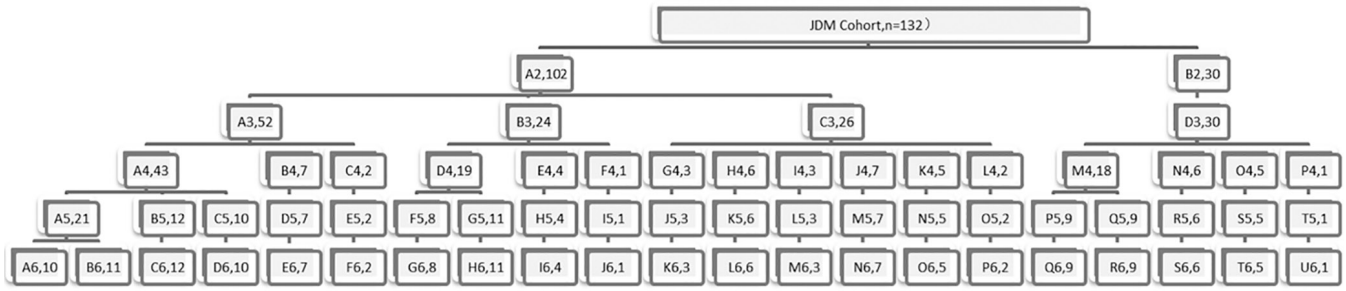

Fig. 2. Agglomerative hierarchical clustering of the132 dermatomyositis patients based on categorical principal components analysis. Here, we present the process of combination from 21 clusters to 1 cluster. The number in the parenthesis indicates the number of patients included in each cluster.

tribution of laboratory data and myositis autoantibodies, which indicated that multidimensional assessment of clinical manifestations is valuable and urgently needed in JDM patients.

In our study, a PCA-based cluster analysis of variables was applied, resulting in a dendrogram in which variables with similar distribution patterns were categorised into the same groups. To detect critical features and reduce the dimensionality of the original variables, PCA was selected as a pre-processing method for cluster analysis. First, as an autoimmune disease, JDM is prominently characterised by multiple-system damage and heterogeneity of symptoms. Some symptoms are less prevalent but significant in the clinical practice. With the ability to maintain the integrity of data, PCA outstands other pre-processing methods, including factor analysis (7), PCA (8) and association analysis (9), and ensures that these less prevalent symptoms are not missed due to methodological flaws. Second, PCA was conducive to effectuate independence of variables and eliminate noisy variables. Independence of the variables, as a prerequisite

for cluster analysis, was a critical factor for the reliability of clustering (10). Clinically, the original variables were limited in independence, which led to unsatisfactory results by direct cluster analysis (11). The results of our study also indicated that PCA-based cluster analysis would be appropriate for subgrouping JDM, a heterogeneous autoimmune disease.

In our study, the 4 groups identified were consistent with some specific subtypes referred to in previous studies and criteria. The characteristics of cluster B (JDM with vasculitis) and cluster C (hypermyopathic JDM) were in accordance with the 1975 classification criteria for DM proposed by Bohan and Peter (4, 5), as well as with Sontheimer's standalone classification criteria for defining amyopathic DM (12).

The presence of various autoantibodies can often be observed in JDM, contributing to the classification, diagnosis, and emergence of particular comorbidities. According to previous studies, anti-Mi2 is associated with classic skin features such as heliotrope rash, Gottron papules, shawl sign, V-sign, photosensitivity, and cuticular overgrowth (13); anti-MDA5 is associated with ulcerations over areas such as lateral nailfolds and elbows, and Gottron papules, oral mucosal pain, tender palmar papules, and ILD (subacute or rapidly progressive) (14); anti-TIF1γis most frequently observed in patients with palmar hyperkeratotic papules, malignancy, and psoriasis-like lesions (15); anti-NXP2 is associated with peripheral oedema, dysphagia, myalgia, calcinosis, and malignancy (16); and anti-Jo1 is associated with antisynthetase syndrome and mechanic's hands (17, 18), while anti-PM-Scl100 is most frequently observed in patients with Raynaud's phenomenon (RP) and arthritis (19, 20). In our study, we found that anti-MDA5, anti-PMScl100, anti-Jo1, and Anti-Mi2 showed significant differences across the 4 identified clusters ($p<0.05$), which were consistent with their clinical characteristics: anti-Jo1 ranked the highest positive rate in cluster D (JDM patients with skin involvement), anti-PM-Scl100 was detected most frequently in cluster B (JDM patients with clinical manifestations of vasculitis including RP) and cluster A (JDM patients with arthralgia and intense inflammation), and anti-MDA5 had the highest positive rate in cluster A (JDM patients with arthralgia and intense inflammation including cough and fever). Although anti-NXP2 failed to show a significant difference, it showed the highest positive rate in cluster B (JDM patients with clinical manifestations of vasculitis including calcinosis) and cluster C (hypermyopathic JDM patients). Therefore, the results of our study confirm the findings of previous studies.

Besides autoantibodies, our results also indicate that the distribution of clinical manifestations is in accordance with previously reported data. Through PCA-based cluster analysis, variables with similar distribution patterns were classified into the same group. Arthralgia and fever were in the same cluster; myalgia, hoarseness, muscle weakness, Raynaud's phenomenon and periungual

Table II. Clinical characteristics of 132 patients with juvenile dermatomyositis according to the clusters identified using principal component analysis-based cluster analysis.

	Group A (n=52)	Group B (n=24)	Group C (n=26)	Gourp D (n=30)	*p*-value
Demographics					
Female (%)	26 (50.0)	14 (58.3)	14 (53.8)	13 (43.3)	0.726
Age at onset[a], years	9.1 (3.2)	7.8 (2.5)	7.5 (3.4)	6.8 (3.6)	0.045*
Course of disease[b], months	10.1 (7.2)	11.4 (3.5)	14.0 (3.7)	9.7 (8.1)	0.634
Clinical features					
Heliotrope rash (%)	42 (80.8)	14 (58.3)	17 (68.0)	24 (80.0)	0.031*
Gottron sign (%)	40 (76.9)	16 (66.7)	20 (80.0)	23 (76.7)	0.688
Muscle weakness (%)	23 (44.2)	3 (12.5)	1 (4.0)	9 (30.0)	0.001*
Myalgia/muscle tenderness (%)	9 (17.3)	5 (20.8)	2 (8.0)	5 (16.7)	0.610
Blephoraedema (%)%	5 (9.5)	4 (16.7)	4 (15.4)	8 (26.7)	0.249
Calcinosis cutis (%)	7 (13.5)	1 (4.2)	0 (0.0)	2 (6.7)	0.165
Digital ulcer (%)	1 (1.9)	1 (4.2)	0 (0.0)	1 (3.3)	0.762
Fever (%)	21 (40.4)	10 (41.7)	4 (19.2)	10 (33.3)	0.036*
Raynaud's phenomenon (%)	1 (1.9)	0 (0.0)	2 (7.7)	0 (0.0)	0.169
Cough (%)	16 (30.8)	9 (37.5)	6 (23.1)	2 (6.7)	0.024*
Periungual telangiectasia (%)	11 (30.8)	3 (12.5)	8 (30.8)	4 (13.3)	0.307
Arthritis/arthralgia (%)	16 (30.8)	8 (33.3)	5 (19.2)	4 (13.3)	0.220
Dysphagia (%)	5 (9.6)	1 (4.2)	2 (7.7)	4 (13.3)	0.700
Choking (%)	7 (13.5)	2 (8.3)	2 (7.7)	4 (13.3)	0.825
Hoarseness (%)	3 (5.8)	0 (0.0)	1 (3.8)	2 (6.7)	0.648
Lipsotrichia (%)	3 (5.8)	0 (0.0)	1 (3.8)	2 (6.7)	0.648
Laboratory data					
Creatine kinase level, U/L	137.0 (1423.6)	135.5 (1127.6)	204.5 (1112.8)	180.5 (1345.7)	0.886
ALP level, U/L	97.5 (118.7)	148.0 (163.7)	130.0 (158.7)	124.5 (159.0)	0.025*
Usage of aggressive immunosuppressive therapy	21 (40.4)	20 (83.3)	11 (42.3)	21 (70.0)	0.001*
Antibody					
Mi2	8 (6.1)	1 (4.2)	1 (3.8)	8 (26.7)	0.011*
TIFγ	10 (19.2)	3 (12.5)	6 (23.1)	7 (23.3)	0.748
MDA5	11 (21.1)	1 (4.2)	2 (7.7)	2 (6.7)	0.038*
NPX2	10 (19.2)	6 (25.0)	7 (26.9)	7 (23.3)	0.875
SAE1	1 (1.9)	0 (0.0)	0 (0.0)	0 (0.0)	0.673
Ku	5 (9.6)	1 (4.2)	2 (7.7)	0 (0.0)	0.343
PMScl100	2 (3.8)	4 (16.7)	1 (3.8)	0 (0.0)	0.044*
PMScl75	3 (5.8)	1 (4.2)	2 (7.7)	3 (10.0)	0.835
Jo1	0 (0.0)	0 (0.0)	0 (0.0)	3 (10.0)	0.016*
SNP	5 (9.6)	1 (4.2)	1 (3.8)	1 (3.3)	0.592
PL7	2 (3.8)	1 (4.2)	1 (3.8)	0 (0.0)	0.707
PL12	0 (0.0)	1 (4.2)	0 (0.0)	0 (0.0)	0.522
EJ	1 (1.9)	0 (0.0)	0 (0.0)	0 (0.0)	1.000
OJ	1 (1.9)	3 (12.5)	0 (0.0)	1 (3.3)	0.090
RO52	12 (23.1)	11 (45.8)	7 (26.9)	14 (46.7)	0.073

[a]Values are expressed as medians (interquartile ranges).
[b]Values are expressed as the mean (standard deviation).

telangiectasia were also integrated together, shedding light on homologous pathological mechanisms among heterogenous symptoms.

Cluster A (JDM patients with arthralgia and intense inflammation) is characterised by prominent symptoms of active inflammation, including higher WBC in peripheral blood, fever, and arthralgia. Cough is also categorised into cluster A, indicating potential lung involvement. However, cluster A showed less muscle involvement and demonstrated more features of amyopathic dermatomyositis (ADM) (21). Anti-MDA5 was with the highest positive rate in this cluster, which partially explains such a distribution. The clinical features of ADM patients with positive anti-MDA5 antibody have been reported previously and matched with our findings (22, 23). With aggressive progression of ILD, the prognosis is reported to be unfavourable, with a 40% mortality rate (24).

Cluster B patients (JDM patients with clinical manifestations of vasculitis) tend to be conspicuous due to symptoms of vasculitis, including Raynaud's phenomenon, digital ulcer, and periungual telangiectasia. Calcinosis cutis, which is more commonly found in children and adolescents, is also included in this cluster. Raynaud's phenomenon is one of the most prevalent symptoms in the mixed connective tissue disease. Digital ulcer and periungual telangiectasia are often found in systemic sclerosis (SSc). The highest positive rate of anti-PM-Scl100 in cluster B was in accordance with the feature of variable distribution. Anti-PM-Scl100 was generally found in patients with PM, DM, SSc, and other connective diseases and most frequently found in overlap syndromes of SSc with PM or DM (24-26). The distribution pattern of variables in cluster B indicated other accompanying CTD. Though with minimal muscle involvement, cluster B showed the maximal rate of aggressive immunosuppressive therapy (83.3%), which may be due to additional immunosuppressive treatment required for the other CTD gathered in cluster B.

Symptoms related to myopathy predominate in cluster C (hypermyopathic JDM patients) and include choking, hoarseness, dysphagia, muscle weakness, and myalgia. Cluster C also showed a relatively high rate of aggressive immunosuppressive therapy (42.3%), and the CK level ranked the first among the 4 clusters despite an insignificant difference (median of CK level was 204.5 U/L, p=0.886). The positive rate of anti-NXP2 was the highest in this cluster, although it failed to show a significant difference, which was consistent with the clinical manifestations.

Cluster D (JDM patients with skin involvement) was characterised by skin involvement and blephoraedema. The positive rate of Anti-Mi2 took the lead of the 4 clusters, which explains the distribution pattern, as anti-Mi2 is closely associated with skin involvement (13).

We also found that serum ALP levels were significantly different among the 4 clusters, and cluster B had the highest median level of serum ALP of 148.0 U/L. As an enzyme detected in osteoblasts in the 1980s, ALP demonstrates higher plasma activity with active bone turnover (27). Moreover, ALP represents a direct indicator of vascular calcification (28-30). As calcinosis cutis was also included in cluster B, the highest level of ALP seems to be a reflection of calcium and phosphorus metabolism in JDM patients and is worthy of further exploration.

There are also some limitations to our study. As a retrospective study, the identified 4 clusters should be validated through a long-term prospective follow-up. We are planning to conduct a prospective study on the basis of the current work and explore specific subtypes including ILD in cluster A, accompanied CTD in cluster B, or malignancy in clusters C and D. Besides, independent cohorts with larger sample scales are also necessary to validate the reliability of the classification described in our study.

Hereby, we applied cluster analysis to a cohort of JDM patients for the first time and explored the intrinsic association between specific antibodies and clusters of clinical symptoms. The study included 132 JDM patients with myositis antibodies measured, making it possible to distinguish diverse phenotypes and demonstrate potential disease pathogenesis. The cluster analysis identified 4 subgroups, all of which fit well with the context of previous studies and literature. Novel subgroups of clinical features were also presented for further exploration. These subgroups may contribute to individualised treatments and improved patient prognosis in the future.

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