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ORIGINAL RESEARCH

Clinical and Molecular Insights Into Anti-MDA5 Antibody-Positive Dermatomyositis: A Single-Center Retrospective and Transcriptomic Study

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Objective: To comprehensively characterize clinical features, diagnostic challenges, and prognostic biomarkers of anti-MDA5 antibody-positive dermatomyositis (MDA5-DM), incorporating transcriptomic analysis to elucidate underlying molecular mechanisms.

Methods: We conducted a retrospective analysis of 29 MDA5-DM patients, collecting detailed clinical and laboratory data. Prognostic factors were identified using LASSO regression, validated by Cox proportional hazards and Kaplan-Meier survival analyses. Public transcriptomic dataset (GSE143323) was analyzed to identify differentially expressed genes and enriched immune pathways.

Results: Patients exhibited a high misdiagnosis rate (62.1%) and prevalent interstitial lung disease (96.6%), with 41.4% developing rapidly progressive ILD (RP-ILD). Serum KL-6 level emerged as an independent predictor of mortality (HR=2.96, $p<0.01$). Transcriptomic profiling revealed upregulation of IL-17, Toll-like receptor, and cytokine–receptor interaction pathways.

Conclusion: MDA5-DM presents formidable diagnostic challenges with high misdiagnosis rates and substantial mortality risk predominantly driven by RP-ILD. Serum KL-6 represents a robust, clinically applicable prognostic biomarker warranting integration into risk stratification protocols. Transcriptomic findings illuminate critical immune-inflammatory cascades, particularly cytokine networks and IL-17 signaling, offering mechanistic insights and potential therapeutic targets. Future multicenter prospective studies are essential to validate these biomarker findings and develop composite prognostic models incorporating clinical, radiographic, and molecular parameters.

Keywords: Anti-MDA5 dermatomyositis, rapidly progressive interstitial lung disease, prognostic biomarkers, KL-6, transcriptomics, immunopathogenesis

Introduction

Dermatomyositis (DM) represents a heterogeneous group of autoimmune inflammatory myopathies characterized by varying involvement of skeletal muscles, skin, and multiple organ systems.[1] Among the recognized myositis-specific autoantibodies, anti-melanoma differentiation-associated gene 5 (MDA5) antibody-positive dermatomyositis (MDA5-DM) has recently garnered substantial clinical and research interest due to its distinct clinical phenotype and poor prognosis.[2]

MDA5-DM is predominantly characterized by rapidly progressive interstitial lung disease (RP-ILD), which frequently precipitates severe respiratory failure and carries a high short-term mortality rate.[3,4] Early in its clinical course, however, MDA5-DM often manifests with nonspecific symptoms such as low-grade fever, dry cough, or subtle cutaneous eruptions, leading to frequent misdiagnosis as infectious diseases, allergic dermatitis, or other connective tissue disorders. This diagnostic ambiguity results in delayed confirmation and initiation of appropriate immunosuppressive therapy, which may exacerbate disease progression and worsen patient outcomes.

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Serum biomarkers such as Krebs von den Lungen-6 (KL-6) and ferritin, together with the presence of RP-ILD, have been previously identified as important prognostic factors in MDA5-DM.[5–7] Concurrently, advances in high-throughput sequencing techniques, particularly transcriptomics, have significantly expanded our understanding of the underlying immune-mediated mechanisms driving this disease. Emerging evidence implicates signaling pathways involving interleukin-17 (IL-17), Toll-like receptors, and chemokine networks as pivotal contributors to the intense inflammatory milieu and rapid disease progression characteristic of MDA5-DM.[8,9]

Despite these advances, comprehensive investigations that integrate both detailed clinical phenotyping and molecular analyses remain scarce. There is a critical need to elucidate core molecular pathways underlying disease pathogenesis, improve risk stratification of high-risk patients, and inform the development of personalized therapeutic strategies.

In this context, our single-center retrospective study enrolled 29 confirmed MDA5-DM patients to systematically delineate their clinical features, misdiagnosis patterns, and key prognostic factors, with a particular focus on serum KL-6 levels. By leveraging publicly available transcriptomic datasets (GSE143323), we further explored differentially expressed genes and immune-inflammatory pathway enrichments to unravel potential molecular mechanisms contributing to the rapid progression of MDA5-DM. Our findings aim to provide a robust theoretical framework and practical guidance for early diagnosis, precise risk assessment, and tailored immunotherapy in this challenging autoimmune condition.

Materials and Methods

Study Design and Participants

From January 2023 to June 2024, 29 patients diagnosed with MDA5-DM were identified at the Departments of Rheumatology and Immunology, Affiliated Hospital 2 of Nantong University. All participants fulfilled the 2017 EULAR/ACR classification criteria for dermatomyositis and tested positive for anti-MDA5 antibodies via immunoblot assay.[10] Patients with incomplete data or lost to follow-up were excluded. All patients were followed up until December 31, 2024. This study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee of Affiliated Hospital 2 of Nantong University (Approval No. 2024KT426).

Clinical Data Collection

Comprehensive clinical data were extracted from inpatient records, outpatient visits, and telephone follow-ups, encompassing the following domains:

Demographics

Age at onset, sex, and relevant comorbidities such as hypertension and diabetes mellitus.

Initial Presentation and Diagnostic Timeline

Department of initial consultation, preliminary diagnosis, time interval from first visit to definitive diagnosis, and documented misdiagnoses, including the nature of incorrect diagnoses.

Clinical Manifestations

Detailed documentation of dermatologic findings (eg, Gottron's papules, V-sign rash, heliotrope rash, fingertip ulcers), muscular involvement (eg, myalgia, proximal muscle weakness), and respiratory symptoms (eg, dry cough, dyspnea, hypoxemia).

Laboratory Parameters

Complete blood counts including absolute lymphocyte counts; muscle enzymes including creatine kinase (CK) and lactate dehydrogenase (LDH); inflammatory markers such as C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), and serum ferritin; systematic serum tumor-marker testing (CEA, CA-125, CA-199, NSE) was attempted but limited by incomplete data collection across the cohort; antinuclear antibody (ANA), Anti-extractable nuclear antigen

antibodies (ENA), anti-MDA5 antibody titers graded semi-quantitatively (+, ++, +++); and Krebs von den Lungen-6 (KL-6) levels.

Radiologic and Pulmonary Function Assessment

High-resolution computed tomography (HRCT) was reviewed independently by two board-certified thoracic radiologists blinded to clinical data. Each scan was classified according to the predominant interstitial-lung-disease pattern: (i) nonspecific interstitial pneumonia-like (NSIP-like), (ii) organizing pneumonia-like (OP-like), (iii) usual interstitial pneumonia-like (UIP-like), (iv) diffuse alveolar damage (DAD), or (v) mixed/overlapping.

Treatment Regimens and Outcomes

Details on glucocorticoid and immunosuppressant use, occurrence of major adverse events such as respiratory failure, mortality, and survival status at latest follow-up.

Definitions

Anti-MDA5 Antibody Detection

Anti-MDA5 antibodies were detected via line immunoblot assay, with antibody levels classified as +, ++, or +++ according to band intensity.

Rapidly Progressive ILD (RP-ILD)

RP-ILD was defined as the occurrence of any of the following within 3 months of respiratory symptom onset:Acute and progressive worsening of dyspnea requiring hospitalization or supplemental oxygen,Pulmonary function impairment, evidenced by a decline of >10% in forced vital capacity (FVC) or >15% in diffusion capacity for carbon monoxide (DLCO),An increase of >20% in the extent of interstitial abnormalities on HRCT,Arterial blood gas analysis indicating respiratory failure or a decrease in partial pressure of oxygen (PaO_2) exceeding 10 mmHg (1 mmHg = 0.133 kPa). Alternative causes, such as severe infection, were excluded.[11,12]

Missing Data Management

For minor missingness in KL-6 measurements (n=4), multiple imputation was implemented incorporating covariates including age, sex, and RP-ILD status to reduce bias.

Statistical Methods

Data Preprocessing

All variables underwent rigorous data encoding and quality control. Continuous variables conforming to normal distribution were described as mean ± standard deviation (eqn ± s), while non-normally distributed data were represented by median and interquartile range (IQR). Categorical variables were expressed as counts and percentages. Missing data, comprising less than 10% of the dataset, were addressed via multiple imputation using the "mice" package in R to mitigate potential biases due to incomplete observations.

Transcriptomic Differential Expression Analysis

To elucidate molecular abnormalities in immune-inflammatory pathways associated with MDA5-DM, publicly available RNA-sequencing data (GSE143323) were obtained from the Gene Expression Omnibus (GEO). Extensive literature searches confirmed that GSE143323 remains the most comprehensive and well-characterized MDA5-DM transcriptomic dataset available in public repositories. The dataset included muscle tissue samples from 36 dermatomyositis patients and 20 healthy controls. After performing quality assessments and excluding outliers with aberrant sequencing depth or expression profiles, raw data were normalized via the normalizeBetweenArrays function in R. Differentially expressed genes (DEGs) were identified using the limma package with thresholds set at $|\log_2 \text{ fold change}| \geq 1$ and p-value < 0.05, followed by adjustment for multiple comparisons using the Benjamini-Hochberg false discovery rate method. Functional enrichment analyses for Gene Ontology (GO) terms and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways were conducted using

the clusterProfiler package, considering p.adjust < 0.05 as statistically significant. Visualization of results employed volcano plots, bubble plots, and bar charts.

Survival Analysis and Prognostic Modeling

LASSO Regression

Candidate prognostic variables, including KL-6, ferritin, and RP-ILD status, were incorporated in LASSO regression models. The optimal regularization parameter (λ) was selected via 10-fold cross-validation to minimize overfitting and collinearity.

Univariate Cox Proportional Hazards Regression

Prognostic variables highlighted by LASSO were further analyzed in univariate Cox models to estimate hazard ratios (HR) with 95% confidence intervals (CI), assessing their association with all-cause mortality or severe respiratory failure.

Kaplan–Meier Survival Analysis

Study population was stratified based on serum KL-6 levels and the presence of RP-ILD; survival curves were generated and compared using Log rank tests. Statistical significance was defined as two-sided $p < 0.05$.

Software and Statistical Tools

Data imputation was performed with the mice package in R version 4.4.3. LASSO regression was conducted using the glmnet package. Survival analyses and Kaplan-Meier plots were generated using SPSS version 26.0. All tests were two-tailed, with p-values less than 0.05 considered statistically significant.

Results

Demographic Characteristics and Diagnostic Challenges

A total of 29 patients diagnosed with MDA5-DM were enrolled in the study. The cohort had a mean age of 52.1 ± 13.6 years, with females accounting for 55.2% (Table 1). Fever was observed in 65.5% of patients, paralleled by an equal proportion (65.5%) presenting documented infections. Notably, 65.5% of patients tested positive for the anti-Ro-52 antibody. During their clinical course, a majority (79.3%) received Janus kinase (JAK) inhibitor therapy.

Among these patients, initial misdiagnosis was common, affecting 18 individuals (62.1%). The most frequent misclassifications included pulmonary infections, idiopathic interstitial pneumonia, and various inflammatory dermatologic diseases, underscoring the diagnostic complexity of early-stage MDA5-DM. The median diagnostic delay was 37 days (interquartile range: 8 to 96 days), potentially compromising timely management and prognosis (Table 2).

Clinical Manifestations and Pulmonary Involvement

ILD was nearly ubiquitous, identified in 28 of 29 patients (96.6%), with RP-ILD observed in 12 patients (41.4%). Dominant respiratory symptoms encompassed dry cough, dyspnea, and hypoxemia. HRCT typically revealed ground-glass opacities and/or extensive consolidations. When available, pulmonary function tests demonstrated markedly reduced diffusing capacity. Comparative analysis of inflammatory markers revealed significantly lower lymphocyte counts in RP-ILD patients compared to Conventional ILD (C-ILD) patients ($p = 0.018$), as shown in Figure 1. Additionally, RP-ILD patients demonstrated significantly elevated CRP ($p = 0.039$) and ferritin ($p = 0.0032$) levels compared to those with C-ILD, further supporting the hyperinflammatory nature of rapidly progressive disease (Figure 1). Dermatological manifestations were prevalent in 96.6% of patients, including Gottron's papules, heliotrope rash, and violaceous papules on the dorsal hands. During follow-up, 5 patients (17.2%) succumbed, highlighting the grave prognosis associated with MDA5-DM. Mortality was notably elevated in patients with RP-ILD, indicating a strong association between this subtype and adverse clinical outcomes.

Table 1 Demographic and Clinical Data of the 29 Patients

Characteristics	Value
Number of Patients (n=29)	
Female, n (%)	16 (55.2)
Male, n (%)	13 (44.8)
Age, mean (SD), years	52.07 (13.57)
Fever, n (%)	19 (65.5)
Tumor, n (%)	3 (10.3)
Infection, n (%)	19 (65.5)
ILD Pattern, n (%)	
Conventional ILD (C-ILD)	16 (55.2)
Rapidly progressive ILD (RP-ILD)	12 (41.4)
Muscle involvement, n (%)	8 (27.6)
Anti-MDA5 antibody titers[a], n (%)	
+	8 (27.6)
++	8 (27.6)
+++	13 (44.8)
Anti-Ro-52 antibody positive, n (%)	19 (65.5)
Erythrocyte sedimentation rate (ESR), mean (SD), mm/h	29.69 (18.89)
Serum ferritin (SF), mean (SD), ng/mL	1400.41 (1265.91)
Lactate dehydrogenase (LDH), mean (SD), U/L	315.86 (107.50)
KL-6, mean (SD), U/mL	802.53 (607.42)
D-Dimer, mean (SD), ug/L	1162.31 (1369.34)
JAK Inhibitor Therapy, n (%)	23 (79.3)

Notes: [a]Anti-MDA5 antibody titers (immunoblot): + weak, ++ moderate, +++ strong positivity.

Table 2 Initial Department Visits and Misdiagnosis Profiles (n = 29)

Department	Cases, n (%)	Misdiagnosed, n (%)	Primary Misdiagnoses
Respiratory Medicine	10 (34.5)	8 (80.0)	Pulmonary infection (5), idiopathic interstitial pneumonia (3)
Dermatology	8 (27.6)	5 (62.5)	Allergic dermatitis (1), Systemic lupus Erythematosus (1),Seborrheic dermatitis (1), drug-induced dermatitis (1), Erythema multiforme (1)
Rheumatology	6 (20.7)	2 (33.3)	Other connective tissue diseases (2)
Infectious Diseases	3 (10.3)	2 (66.7)	Fever of unknown origin (1), viral infection (1)
Other Departments	2 (6.9)	1(50)	Miscellaneous (1)
Total	**29 (100)**	**18 (62.1)**	

Survival Analysis and Prognostic Significance of KL-6

LASSO regression analysis identified serum KL-6 level as the predominant prognostic biomarker predicting patient survival (Figures 2). Elevated KL-6 was independently associated with an increased hazard of mortality (hazard ratio [HR] = 2.96, 95% confidence interval [CI]: 1.44–6.15, $p < 0.01$). This association was further supported by Kaplan-Meier survival curves, which demonstrated significantly diminished survival probabilities in patients with high KL-6 compared to those with lower levels (Log rank test, $p < 0.05$) (Figure 3A). Notably, this survival disparity was especially evident in patients diagnosed with RP-ILD (Figure 3B).

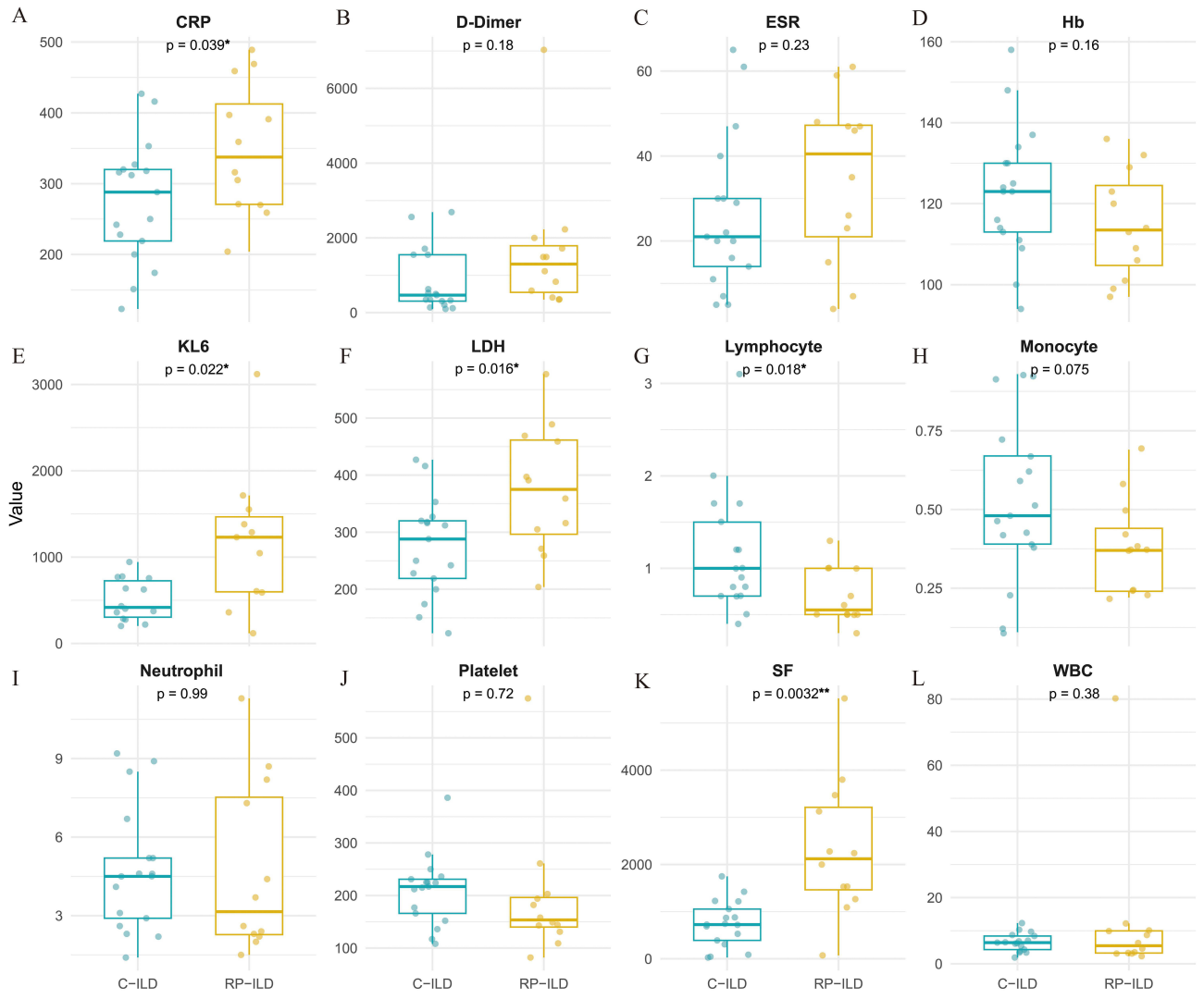

Figure 1 Comparative analysis of inflammatory markers between RP-ILD and C-ILD patients.Variables including CRP (**A**), D-Dimer (**B**), ESR (**C**), Hemoglobin (**D**), KL-6 (**E**), LDH (**F**), Lymphocyte (**G**), Monocyte (**H**), Neutrophil (I), Platelet (**J**), Serum Ferritin (**K**), and WBC (**L**) were compared between RP-ILD (yellow) and C-ILD (blue) groups. Statistical significance determined by Mann–Whitney *U*-test. Significant differences are marked with * ($p < 0.05$) and ** ($p < 0.01$).

Transcriptomic Profiling

Differential Gene Expression Analysis

Transcriptomic data (GSE143323) comprising muscle biopsies from 36 dermatomyositis patients and 20 healthy controls were analyzed. After rigorous quality control and exclusion of outliers, differential expression analysis was performed using the limma and DESeq2 packages. With thresholds set at $|\log_2$ fold change$| \geq 1$ and Benjamini-Hochberg adjusted p-value < 0.05, we identified 182 differentially expressed genes (DEGs), including 141 upregulated and 41 down-regulated (Figure 4).

KEGG Pathway Enrichment

KEGG enrichment analysis revealed significant overrepresentation of multiple immune and inflammatory signaling pathways among the DEGs. The most prominently enriched pathways included cytokine-cytokine receptor interactions (16 genes, p.adjust < 0.01), viral protein interaction pathways (12 genes, p.adjust < 0.01), and chemokine signaling (9 genes, p.adjust < 0.01). Additional significantly enriched pathways comprised IL-17 signaling and Toll-like receptor signaling pathways (Figure 5).

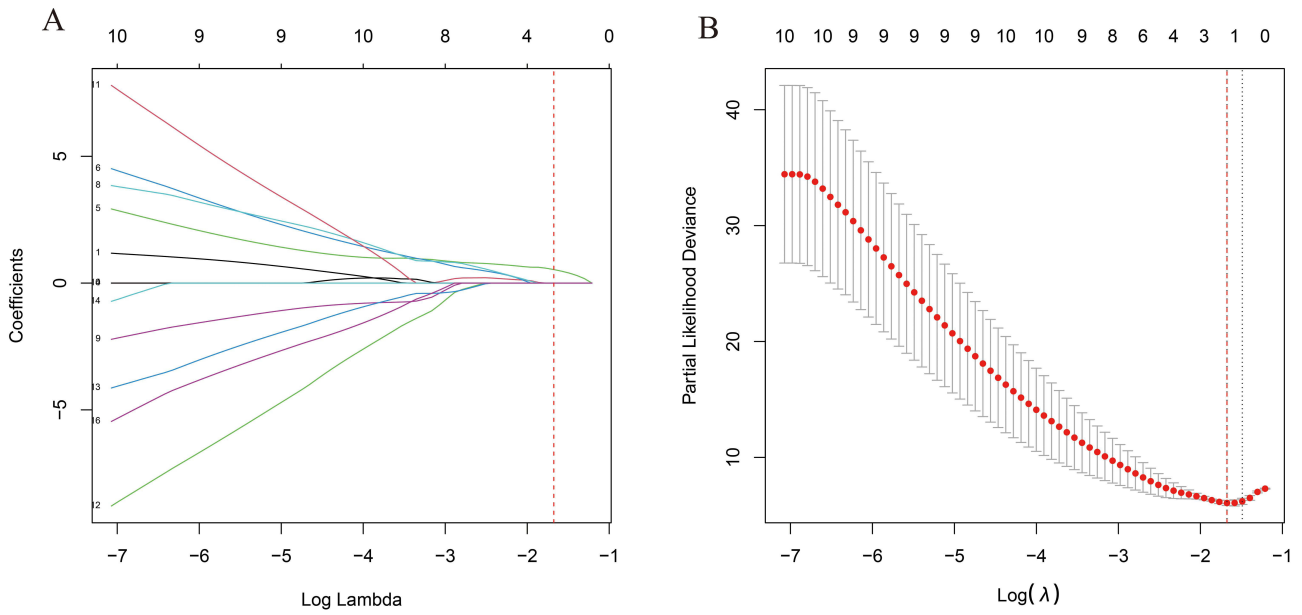

Figure 2 Variable selection by LASSO method. (**A**) Coefficients of all predictors gradually approached zero through 10-fold cross-validation. (**B**) Coefficients of 16 variables were non-zero at the leftmost dashed line (λ = λ.min). Min refers to the minimum value of λ.

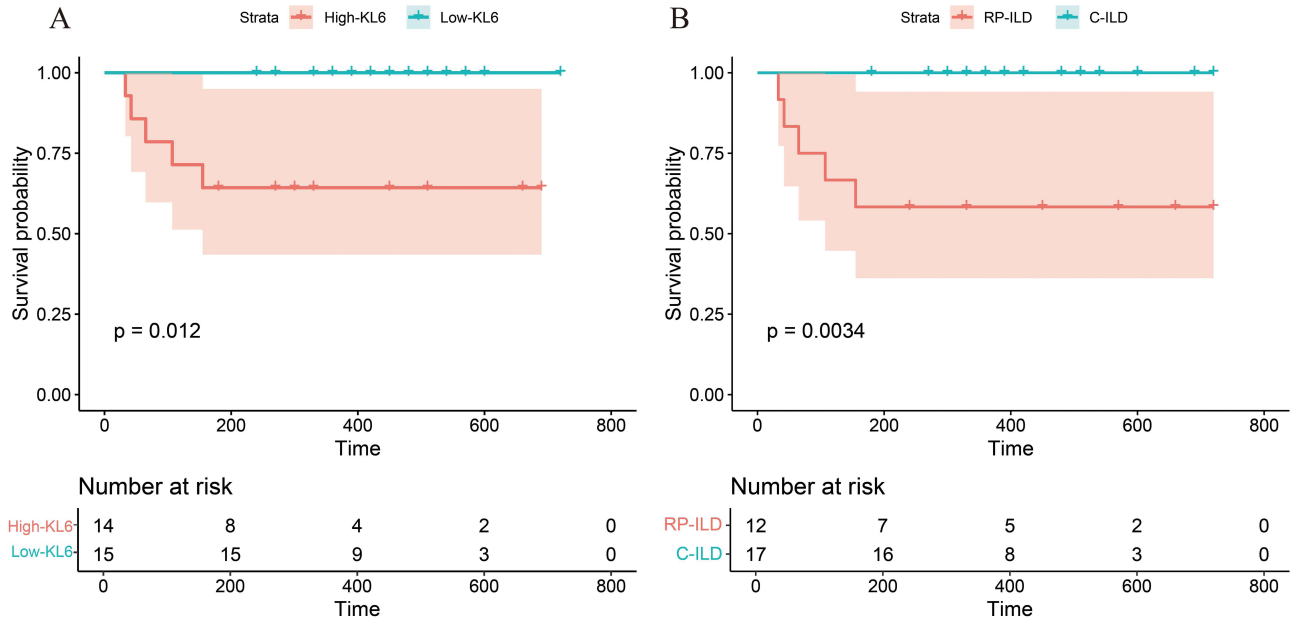

Figure 3 Kaplan-Meier survival curves comparing survival between different groups. (**A**) Survival curves based on high vs low KL6 levels, showing a significant difference with a p-value of 0.012. (**B**) Survival curves comparing RP-ILD and C-ILD groups, with a significant difference observed (p = 0.0034).

Gene Ontology Enrichment

Gene Ontology enrichment analysis indicated significant involvement of biological processes such as neutrophil chemotaxis, T-cell activation, and broader immune responses. Molecular function and cellular component categories highlighted modulation of inflammatory mediator activity and immune receptor localization. Collectively, these transcriptomic findings corroborate the notion of a heightened and coordinated inflammatory milieu underlying the pathogenesis of MDA5-DM (Figure 6).

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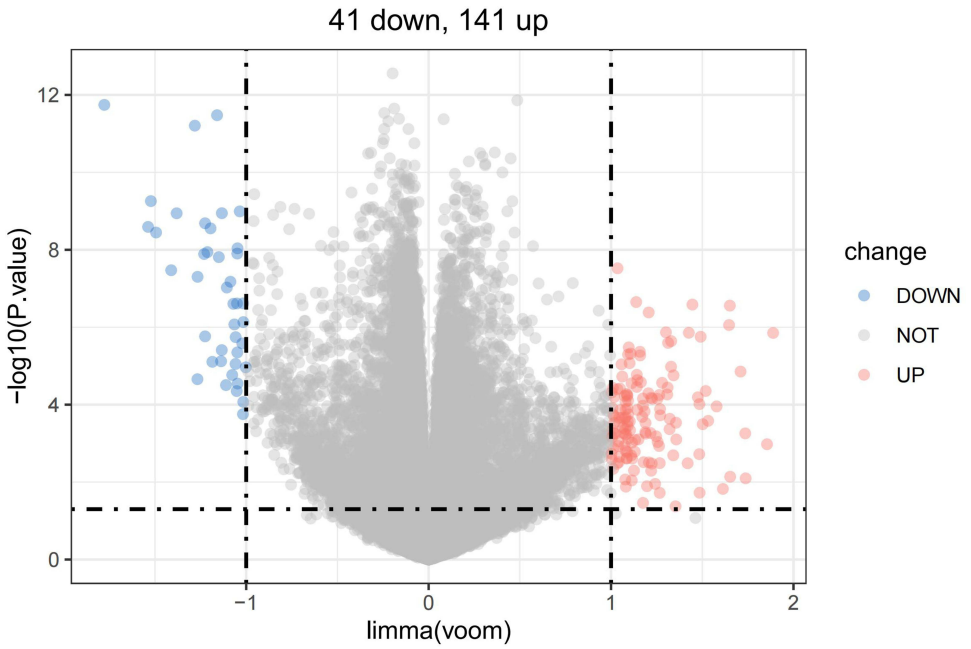

Figure 4 Volcano plot of differential gene expression analysis. Genes with log2 fold change ≥ 1 and p-value < 0.05 are shown in red, indicating significantly upregulated genes. Genes with log2 fold change ≤ −1 and p-value < 0.05 are shown in blue, indicating significantly downregulated genes. Genes with no significant difference are shown in grey.

Discussion

This single-center retrospective study systematically evaluated the clinical characteristics, diagnostic challenges, and prognostic indicators of MDA5-DM, complemented by transcriptomic analyses to explore the underlying immunopathological mechanisms. Our findings highlight a notably high misdiagnosis rate and substantial diagnostic delay, with approximately one-third of patients developing RP-ILD, which was strongly associated with increased mortality.[13] Serum KL-6 emerged as an independent and robust prognostic biomarker, while transcriptomic data revealed significant activation of IL-17, Toll-like receptor, and other cytokine-mediated signaling pathways, shedding light on the molecular drivers of disease aggressiveness.

Diagnostic Challenges and the Importance of Early Recognition

We observed a high misdiagnosis rate of 62.1%, with the majority of patients initially presenting to non-rheumatology specialties. This underscores the heterogeneous and frequently nonspecific clinical manifestations of MDA5-DM, which are often mistaken for pulmonary infections, idiopathic interstitial pneumonia, or inflammatory dermatologic conditions.[14] Such diagnostic inaccuracies contribute to delayed definitive diagnosis and postponement of immunosuppressive therapy initiation, thereby accelerating the onset of RP-ILD and adversely impacting prognosis. Prior studies have similarly underscored the critical importance of minimizing diagnostic delays to improve overall survival.[15] Clinicians should maintain a high index of suspicion for MDA5-DM in patients presenting with respiratory symptoms alongside atypical cutaneous manifestations and promptly perform anti-MDA5 antibody testing to facilitate early diagnosis and timely intervention.Recent studies have highlighted how cutaneous manifestations serve as early indicators of systemic immune dysregulation in inflammatory dermatomyopathies, emphasizing the importance of integrated dermatologic-rheumatologic evaluation in suspected cases. Early recognition of these manifestations is crucial for timely diagnosis and intervention, potentially preventing progression to life-threatening complications such as RP-ILD.

Prognostic Biomarkers and Risk Stratification

Employing LASSO regression modeling, serum KL-6 was identified as the principal prognostic indicator. Subsequent univariate Cox proportional hazards and Kaplan–Meier survival analyses consistently confirmed that elevated KL-6

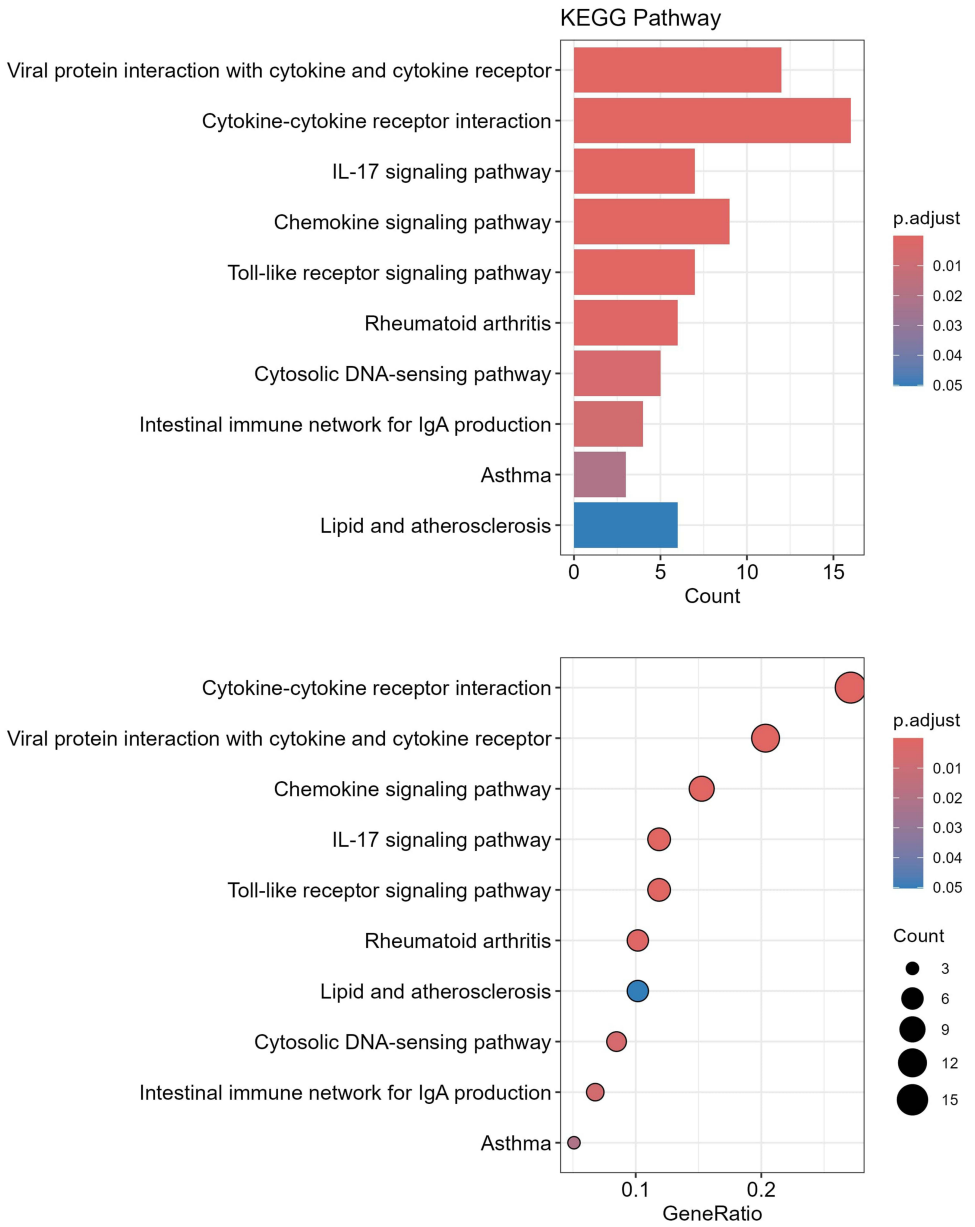

Figure 5 KEGG pathway enrichment analysis of differentially expressed genes.Bar plot showing significantly enriched pathways ranked by adjusted p-value (p.adjust). Dot plot visualizing the same pathways, with dot size representing gene count and color intensity indicating the statistical significance (p.adjust).

levels were strongly associated with increased mortality risk. As a mucin-like glycoprotein expressed by regenerating alveolar type II epithelial cells, KL-6 serves as an established biomarker for alveolar epithelial injury and active pulmonary inflammation.[16] The prognostic significance was particularly pronounced in RP-ILD patients, where elevated KL-6 combined with rapid disease progression showed markedly diminished survival outcomes.

Our comparative analysis revealed distinct inflammatory profiles differentiating RP-ILD from conventional ILD patterns. RP-ILD patients demonstrated significantly lower lymphocyte counts, elevated CRP, and increased ferritin levels, supporting the hyperinflammatory nature of rapidly progressive disease. However, despite previous studies proposing ferritin and other inflammatory markers as prognostic indicators,[17,18] our survival analysis did not demonstrate statistically significant mortality associations, likely reflecting our limited sample size and patient heterogeneity.

The 17.2% mortality rate observed in our cohort underscores the severe clinical trajectory of MDA5-DM, particularly in patients with concurrent RP-ILD and KL-6 elevation. The lymphopenia observed in RP-ILD patients corroborates

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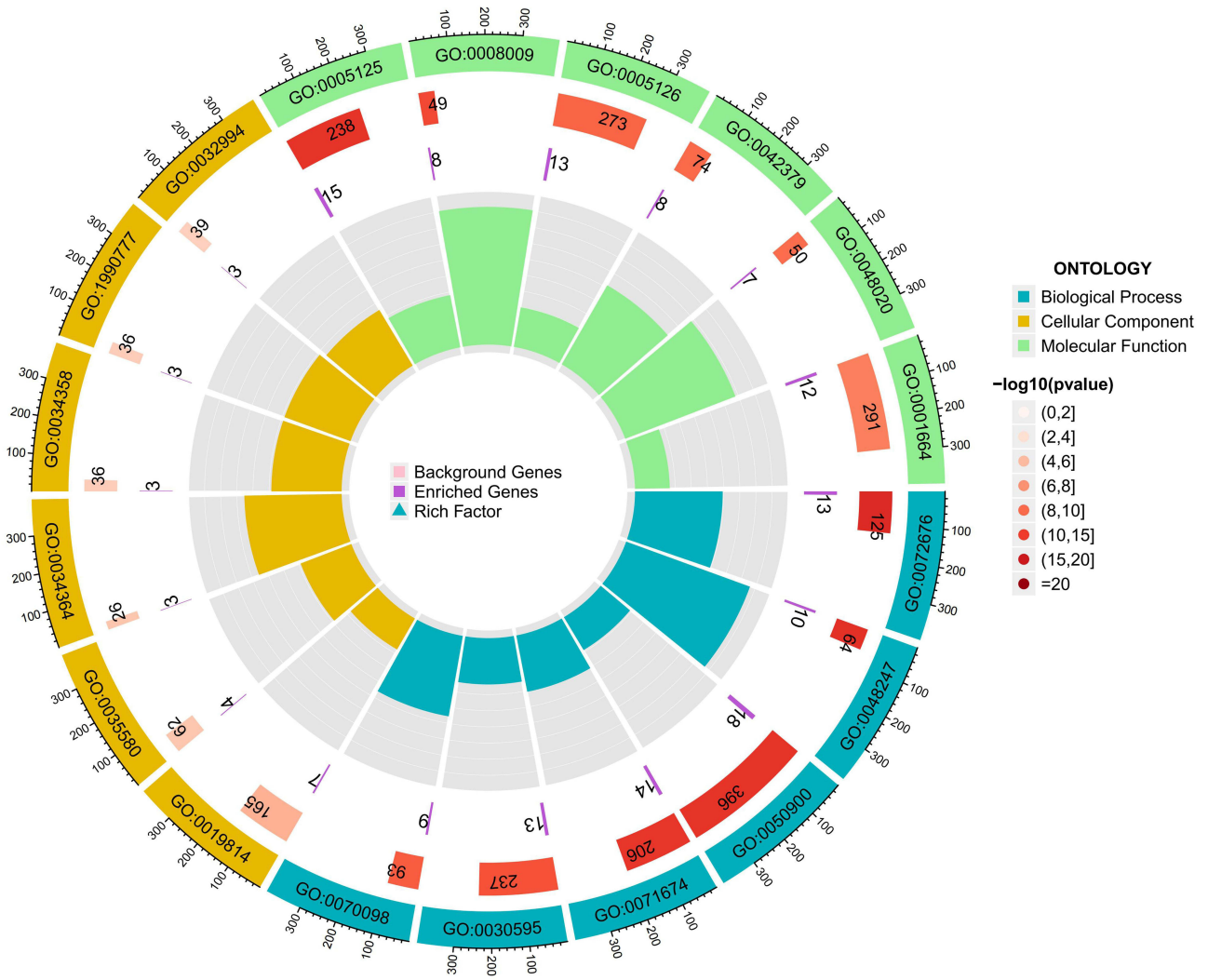

Figure 6 Circular visualization of enriched GO terms. The circle plot displays the top 18 enriched GO terms, categorized into Biological Process (BP), Molecular Function (MF), and Cellular Component (CC).

previous findings establishing lymphocyte count as a severity marker, potentially reflecting the intense systemic inflammatory response characteristic of this phenotype.

Based on these findings, KL-6 measurement should be incorporated into initial evaluation of patients with respiratory symptoms and radiographic pulmonary inflammation when MDA5-DM is suspected. The combination of persistent KL-6 elevation with rising ferritin and progressive HRCT abnormalities may identify highest-risk patients warranting treatment intensification, as serial KL-6 monitoring could provide earlier progression detection than traditional parameters alone. However, definitive threshold values require validation in larger prospective cohorts, and clinical implementation should be guided by institution-specific protocols developed through multidisciplinary specialist consultation. Future research should prioritize developing validated composite scoring systems integrating KL-6 with clinical, radiographic, and additional biomarker parameters to optimize personalized MDA5-DM management strategies.

Insights From Transcriptomic Profiling and Molecular Pathogenesis

Our integrative transcriptomic analysis of muscle biopsy samples revealed a coordinated activation of immune-inflammatory networks that may explain the aggressive phenotype characteristic of MDA5-DM. The analysis identified three interconnected pathways that form a self-perpetuating inflammatory circuit underlying disease pathogenesis.

The predominant enrichment of cytokine-cytokine receptor interactions reflects the systemic hyperinflammatory state characteristic of MDA5-DM.[19,20] Among these cytokine networks, IL-6 plays a particularly central role in orchestrating inflammatory cascades and has been recognized for its central role in various inflammatory conditions,[21] with particular relevance to autoimmune pathogenesis. This finding is mechanistically significant as muscle inflammation serves as a central source of circulating inflammatory mediators, contributing to the multi-organ involvement that defines this condition.[22] The extensive cytokine network activation provides a molecular foundation for understanding the severe systemic manifestations observed in MDA5-DM patients.

Concurrently, IL-17 signaling pathway enrichment suggests prominent Th17-mediated inflammatory activation with direct implications for pulmonary pathology. Although our analysis utilized muscle tissue, the IL-17 pathway activation observed likely has broader implications for the devastating RP-ILD phenotype. Emerging evidence demonstrates IL-17's crucial role in pulmonary fibrosis through enhanced neutrophil recruitment and fibroblast proliferation,[23,24] potentially representing a mechanistic link between muscle inflammation and the severe pulmonary manifestations in our cohort.

The enrichment of TLR pathways, particularly involving TLR3/7 components, is mechanistically significant given MDA5's function as a cytoplasmic RNA sensor.[25–27] Enhanced recognition of nucleic acid damage-associated molecular patterns (DAMPs) creates self-amplifying inflammatory circuits that perpetuate both muscle and pulmonary pathology. This mechanism explains the refractory nature of MDA5-DM and its tendency toward rapid progression.

These findings support an integrated pathogenic model where (1) initial MDA5 activation triggers innate immune responses through TLR signaling, (2) this leads to adaptive immune polarization toward Th17 responses, and (3) the resulting cytokine storm amplifies systemic inflammation and tissue damage. This integrated circuit explains why MDA5-DM demonstrates both rapid progression and resistance to conventional immunosuppression.

Our analysis also revealed JAK-STAT signaling pathway enrichment, providing direct therapeutic relevance.[28] Janus kinase (JAK) inhibitors have emerged as promising agents for refractory MDA5-DM, with recent studies demonstrating efficacy in improving pulmonary function and controlling systemic inflammation.[29–31] At our institution, 79.3% of patients received JAK inhibitor therapy, reflecting our center's treatment protocols that incorporate this mechanistic rationale into clinical practice.

The transcriptomic findings support therapeutic strategies that simultaneously target multiple nodes of this inflammatory network through IL-17 signaling inhibition, TLR pathway modulation, and JAK-STAT pathway blockade. While our study was not powered to definitively assess treatment outcomes, the mechanistic insights provide a molecular framework for understanding why multi-target approaches may be necessary for this complex autoimmune condition. However, we acknowledge the limitation of extrapolating muscle-derived molecular signatures to lung pathophysiology and emphasize the need for direct validation in pulmonary tissues or bronchoalveolar compartments.

Future prospective studies and randomized controlled trials are essential to validate these therapeutic targets and optimize combination strategies based on individual molecular profiles, ultimately advancing precision medicine approaches in MDA5-DM management.

Study Limitations and Future Directions

Several limitations should be noted. The retrospective design and relatively small sample size at a single center limit the generalizability and statistical power of our findings. While comprehensive tumor marker analysis was limited by incomplete data collection across our cohort, this represents an acknowledged limitation of our retrospective design. Additionally, although our radiologists identified diverse ILD patterns (NSIP, OP, UIP, DAD, and overlapping patterns), our sample size of 29 patients precluded meaningful statistical analysis across multiple radiographic subgroups. The insufficient statistical power to detect radiographic pattern-biomarker associations limits our ability to provide clinically actionable insights regarding imaging predictors of disease severity or treatment response. Future multicenter studies should prioritize establishing critical radiographic-biomarker correlations to advance precision medicine in MDA5-DM, with particular emphasis on collecting samples stratified by ILD progression status to identify distinct molecular signatures.

Composite Prognostic Score Development

Future research should focus on developing an "MDA5-ILD Risk Score" incorporating KL-6 levels, HRCT patterns, oxygen saturation trajectory, lymphocyte count, and selected gene expression markers. While requiring validation in larger cohorts, such a composite tool could substantially improve personalized treatment strategies and guide therapeutic intensity decisions in clinical practice.

Conclusion

This study reveals that MDA5-DM presents significant diagnostic challenges with a 62.1% misdiagnosis rate and substantial mortality risk predominantly driven by RP-ILD. Serum KL-6 emerged as a robust independent prognostic biomarker, warranting integration into clinical risk stratification protocols. Transcriptomic analysis illuminated critical immune-inflammatory cascades, particularly cytokine networks and IL-17 signaling, offering mechanistic insights and potential therapeutic targets.

These findings establish KL-6 as a clinically applicable prognostic tool while highlighting the need for early recognition and aggressive management of high-risk patients. Future multicenter prospective studies are essential to validate these biomarker findings and develop composite prognostic models incorporating clinical, radiographic, and molecular parameters to advance precision medicine approaches in MDA5-DM.

Data Sharing Statement

The data supporting the findings of this study are available from the corresponding author on reasonable request.

Ethics Declarations

The study was approved by the Ethics Committee of Affiliated Hospital 2 of Nantong University (Approval No. 2024KT426). As this was a retrospective analysis of anonymized clinical data, the requirement for written informed consent was waived by the Ethics Committee. All patient information was de-identified to ensure confidentiality.

Author Contributions

Yunli Ren and Tianqi Wu contributed equally to this work as co-first authors. All authors have made a substantial contribution to the work reported, be it in conception, design, conduct, acquisition of data, analysis and interpretation, or all of these; have been involved in drafting, revising, or critically reviewing the article; have given final approval for the version to be published; have agreed on the journal to which the article will be submitted; and agree to accept responsibility for all aspects of the work.

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Disclosure

The authors declare that they have no competing interests in this work.

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