Pneumocystis jirovecii pneumonia in anti-MDA5-positive dermatomyositis: characterisation, risk factors and prognosis

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Abstract

Objectives

This study aimed to identify risk and prognostic factors of Pneumocystis jirovecii *pneumonia (PJP) in patients with anti-melanoma differentiation-associated gene 5 antibody-positive dermatomyositis (anti-MDA5+DM).*

Methods

We conducted a retrospective cohort study of anti-MDA5+DM patients who underwent metagenomic next-generation sequencing analysis of bronchoalveolar lavage fluid or lung tissue at our center between January 2019 and February 2023. Eligible patients were stratified into PJP+ and PJP- groups based on PJP status. Potential risk factors and prognostic indicators for PJP were analysed using univariate and multivariate logistic regression analysis.

Results

A total of 107 anti-MDA5+DM patients were enrolled, of whom 47 were assigned to the PJP+ group. Multivariate logistic regression analysis revealed older age and high cumulative dosage of glucocorticoids within 3 months preceding PJP diagnosis were independent risk factors for PJP development. Conversely, prophylactic-dose trimethoprim-sulfamethoxazole (TMP/SMZ) was associated with a significantly reduced risk of PJP (all $p<0.05$). The 30-day mortality rate in the PJP+ group was 55.3%. Short disease duration and immunosuppressive therapy exposure, severe hypoxia, extensive radiological interstitial lung disease, moderate to severe acute respiratory distress syndrome, mechanical ventilation were associated with unfavourable prognosis (all $p<0.05$). Glucocorticoids therapy was more frenquently administered in survivors ($p<0.05$).

Conclusions

PJP significantly increases early mortality of anti-MDA5+DM patients. Clinicians should identify high-risk patients early and administer prophylactic-dose TMP/SMZ for PJP prophylaxis.

Key word

pneumonia, pneumocystis, dermatomyositis, high-throughput Nucleotide sequencing, opportunistic infections

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Introduction

Pneumocystis jirovecii, an opportunistic fungal pathogen, has been historically recognised as a frequent cause of life-threatening pneumonia in patients infected with human immunodeficiency virus (HIV), as the most common situation in the past. However, the incidence rate of *Pneumocystis jirovecii* pneumonia (PJP) among patients with autoimmune inflammatory rheumatic disease (AIRD) has also been increasing over the past two decades (1). This epidemiological transition parallels advancements in therapeutic approaches for AIRD, particularly the widespread use of potent immunosuppressive regimens.

Previous epidemiological studies have identified Wegener's granulomatosis as the most common AIRD associated with PJP development, with idiopathic inflammatory myopathies (IIMs) also representing a significant risk group (1, 2). Anti-melanoma differentiation-associated gene 5 antibody-positive dermatomyositis (anti-MDA5+DM) as a particularly severe subtype of IIMs, characterised by hallmark cutaneous manifestations, profound lymphopenia, and rapidly progressive interstitial lung disease (ILD), has received increasing attention due to its high mortality (3, 4). The heightened mortality observed in anti-MDA5+DM patients is multifactorial, among which severe pulmonary inflammation frequently compounded by opportunistic infections representing a key contributor. Lymphopenia and intensive immunosuppressive therapy (IST) creating an conductive to various opportunistic pathogens including PJP (5, 6). Emerging evidence suggests that PJP in dermatomyositis (DM) result in higher mortality rate exceeding those observed in other rheumatic diseases(7). The diagnostic challenge lies in the overlap between PJP and anti-MDA5+DM, both manifesting with fever and ILD on imaging, frequently leading to delayed diagnosis and treatment. Therefore, in this study we aimed to determine the risk factors and prognostic indicators for PJP in anti-MDA5+ DM patients.

Materials and methods

Study population

We conducted a retrospective cohort study of consecutive anti-MDA5+DM patients who underwent metagenomic next-generation sequencing (mNGS) analysis of bronchoalveolar lavage fluid (BALF) or lung tissue at the First Affiliated Hospital of Zhengzhou University between January 2019 and February 2023. Anti-MDA5+DM diagnosis required fulfillment at least one diagnostic criterion: the Bohan and Peter criteria (8), the 2017 EULAR/ACR IIM classification criteria (9) or the 2018 EMNC DM criteria (10) by experienced rheumatologists. Serum anti-MDA5 antibodies were quantified using a commercially available ELISA kit (MBL, Japan) with a cut-off value of 32 U/ml(11). PJP diagnosis required fulfillment all of the following criteria: (i) one or more of the following clinical symptoms: fever, cough, white sputum or dyspnea; (ii) computed tomography (CT) showing ground glass opacity (GGO) and/or consolidation dominated in lungs suggestive of PJP; (iii) positive result for *Pneumocystis jirovecii* by mNGS (12, 13). Patients without etiological evidence or showing a positive sequencing result in absence of clinical symptoms were not fulfilling the PJP diagnostic criteria and sequentially assigned to the PJP- group. The study was conducted in accordance with the Declaration of Helsinki and approved by the Ethical Review Committee of the First Affiliated Hospital of Zhengzhou University (IRB number 2020-KY-194). Informed consent was obtained from all participants or waived by the committee.

Clinical data

We systematically collected the following clinical parameters from medical records: demographical characteristics, clinical characteristics, radiological assessments, laboratory results, treatment regimens and follow-up information. Radiological assessments included GGO, consolidation patterns, cystic changes, pleural effusion (14), and the extent of interstitial involvement via high-resolution CT. Rapidly progressive interstitial lung disease (RPILD)

was defined as previously published (15). We collected immunosuppressive therapy exposure within 3 months preceding PJP diagnosis, including the treatment of glucocorticoids (GCs) (recorded as prednisone equivalents), conventional synthetic, biological and targeted synthetic disease-modifying anti-rheumatic drugs (DMARDs). High cumulative dosage of GCs was defined as the administration of prednisone at least 30mg daily for at least 28 consecutive days (or equivalent GCs) within 3 months preceding PJP diagnosis. At-risk dose of GCs were defined as prednisone at least 20mg (or equivalent GCs). Trimethoprim-sulfamethoxazole (TMP/SMZ) prophylaxis was defined as continuous administration for at least 28 consecutive days prior to PJP diagnosis.

Statistical analysis

All statistical analyses were performed using IBM SPSS software (version 23.0, Armonk, NY, USA). Data are expressed as mean ± standard deviation for normally distributed variables or median (interquartile range) for non-normally distributed variables, as appropriate. Comparisons between groups were conducted using independent-sample t-tests for normally continuous variables, Mann-Whitney U tests for non-normally continuous variables, and Chi-square or Fisher's exact tests for categorical variables. Univariate logistic regression was performed to identify potential risk factors associated with PJP occurrence and 30-day mortality. Variables demonstrating significance ($p<0.05$) in univariate analysis were entered into multivariate logistic regression models to identify risk factors associated with PJP occurrence using back ward stepwise elimination. Results are reported as odds ratios (OR) with 95% confidence intervals (CI). Predictive performance of significant multivariate models was assessed by receiver operating characteristic (ROC) curve analysis. Optimal cutoff values were determined using Youden's index methhod. For all analyses, two-tailed p-values <0.05 were considered statistically significant.

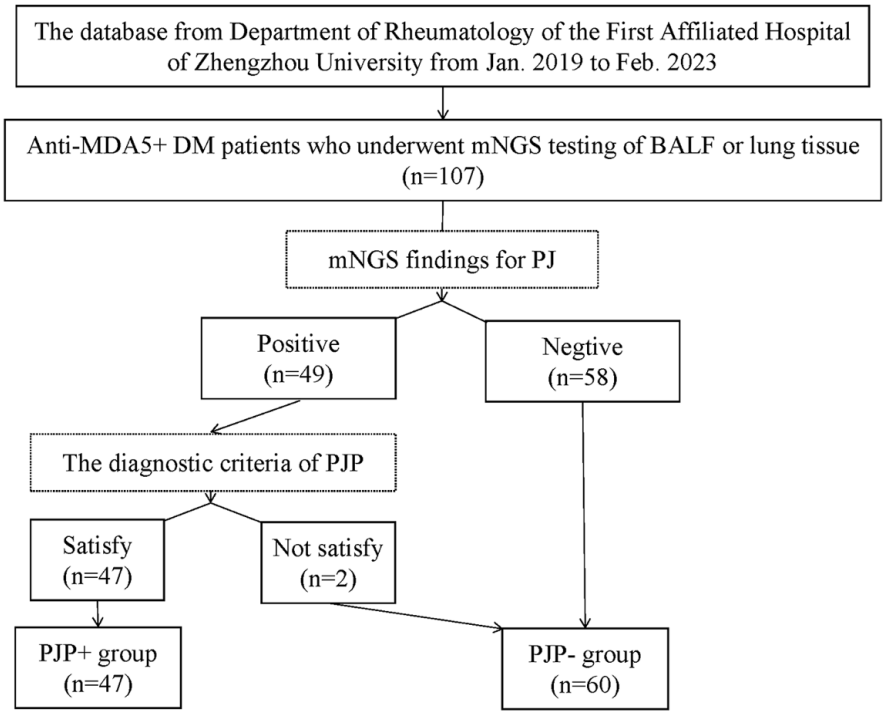

Fig. 1. Flow-chart of this study.
Anti-MDA5+DM: anti-melanoma differentiation-associated gene 5 antibody-positive dermatomyositis; mNGS: metagenomic next-generation sequencing; BALF: bronchoalveolar lavage fluid; PJP: *Pneumocystis jirovecii* pneumonia.

Results

Clinical characteristics of anti-MDA5+ DM patients with PJP

During the four-year study period, we identified 107 anti-MDA5+DM patients who underwent mNGS analysis of BALF or lung tissue at our center. All participants were HIV-negative. Among those, 49 patients showed a positive result of *Pneumocystis jirovecii*. After applying rigorous diagnostic criteria, two cases were excluded due to insufficient clinical evidence of PJP and assigned to the PJP- group. This resulted in 47 PJP+ cases and 60 PJP- controls (Fig. 1). We summarised and compared the clinical features of anti-MDA5+DM patients with or without PJP (Table I). The median age of patients in the PJP+ group was 55 years old at the diagnosis of PJP, which was significantly higher than that in the PJP- group ($p=0.011$). In terms of comorbidity, the prevalence of diabetes was higher in the PJP+ group ($p=0.039$).

In the PJP+ group, the disease course of DM was shorter than the PJP- group, ($p=0.049$) and fever, low oxygenation index, and RPILD ($p<0.001$, $p=0.007$, $p=0.01$, respectively) were more commonly. For treatment, 12 and 14 patients received neither GCs nor DMARDs in the PJP+ and PJP- groups, respectively. Compared with the PJP- group, the cumulative dosage of GCs was higher in the PJP+ group ($p=0.032$). Prophylactic-dose TMP/SMZ was more frequently administered to patients in the PJP- group ($p=0.014$). There was no significant difference in DMARDs use between the groups.

Risk factors related to PJP occurrence

To identify risk factors for PJP occurrence, we used the logistic regression model and included factors with $p<0.05$ in dichotomous comparisons and those considered clinically significant (Table I). In univariate analysis, possible risk factors included older age [adjusted odds ratio (OR)=1.039, 95% CI of 1.004, 1.075], combined diabetes (adjusted OR=2.381, 95% CI of 1.035, 5.477), short disease course of DM (less than 6 months, adjusted OR=0.341, 95% CI of 0.123, 0.946) and high cumulative dosage of GCs (adjusted OR=2.545, 95% CI of 1.074, 6.032). Prophylactic-dose TMP/SMZ

Table I. Comparisons of clinical characteristics between PJP+ and PJP- anti-MDA5+DM patients.

Variables	PJP+ group (n=47)	PJP- group (n=60)	*p*-value
Age, median (IQR)	55 (15)	51 (11)	**0.011**
Male gender, n %	23 (48.9)	26 (43.3)	0.564
Smoking history, n %	9 (19.1)	14 (23.3)	0.601
Disease course of DM, median (IQR), months	1 (2)	3 (5)	**0.049**
Clinical manifestations			
Dyspnoea, n %	35 (74.5)	43 (71.7)	0.746
Specific rash, n % [a]	25 (53.2)	31 (51.7)	0.784
Cough, n %	28 (59.6)	35 (58.3)	0.897
Chest pain, n %	0 (0)	3 (5.0)	0.12
Fever, n %	34 (72.3)	22 (36.7)	**<0.001**
RPILD, n %	28 (59.6)	21 (35.0)	**0.011**
Oxygenation index, median (IQR)	190 (210)	306 (436)	**0.007**
Comorbidities, n %			
Diabetes	20 (42.6)	14 (23.7)	**0.039**
Hypertension	13 (27.7)	14 (23.3)	0.609
Thyroid disorder	4 (8.5)	7 (11.7)	0.594
Malignancy	2 (4.3)	1 (1.7)	0.421
Chronic liver disease	5 (10.6)	2 (3.3)	0.129
Chronic heart disease	3 (6.4)	5 (8.3)	0.703
Treatment, 3 months before pathogen detection			
Duration of IST, median (IQR), days	30 (55)[b]	57 (130)[c]	0.446
Steroid used, n %	35 (74.5)	46 (76.7)	0.751
Daily dosage, median (IQR), mg/day [d]	29.0 (56.5)[b]	10.0 (43.0)[c]	0.178
Cumulative time at-risk, median (IQR), days[e]	23 (61)[b]	6 (27)[c]	0.166
High cumulative dosage, n %[f]	19 (40.4)[b]	12 (20.0)[c]	**0.032**
csDMARDs used, n %	21 (44.7)[b]	26 (43.3)[c]	0.772
Tac, n %	9 (19.1)[b]	16 (26.7)[c]	0.362
CsA, n %	4 (8.5)[b]	4 (6.7)[c]	0.719
MTX, n %	2 (4.3)[b]	0 (0)[c]	0.107
CTX, n %	9 (19.1)[b]	13 (21.7)[c]	0.749
HCQ, n %	4 (8.5)[b]	4 (6.7)[c]	0.719
Combination of csDMARDs, n %	5 (10.6)[b]	8 (13.3)[c]	0.672
Bio/target DMARDs used, n %	10 (21.3)[b]	10 (16.7)[c]	0.731
TOF, n %	10 (21.3)[b]	8 (13.3)[c]	0.276
TCZ, n %	0 (0)[b]	2 (3.3)[c]	0.200
TMP/SMZ prophylaxis (prophylacic-dose), n %	7 (14.9)	22 (36.7)	**0.014**
Clinical outcomes			
One-month mortality, n %	26 (55.3)	18 (30.0)	**0.008**

[a]Specific rash included Heliotrope rash, Gottron's sign, mechanic's hand, and digital tip ulceration. [b]Data available for 46 patients. [c]Data available for 55 patients. [d]Average daily dose of GCs used in 28 days before the diagnosis of PJP and expressed as the prednisone equivalent dose. [e]Days with prednisone used at least 20 mg/day or equivalent GCs within 3 months preceding the PJP diagnosis. [f]Prednisone used at least 30mg daily for at least 28 days or equivalent GCs within 3 months preceding PJP diagnosis.

Anti-MDA5+DM: anti-melanoma differentiation-associated gene 5 antibody-positive dermatomyositis; Bio/target DMARDs: biological/targeted synthetic disease modifying anti-rheumatic drugs; csDMARDs: conventional synthesized disease modifying anti-rheumatic drugs; CsA: cyclosporine A; CTX: cyclophosphamide; DM: dermatomyositis; GCs: glucocorticoids; HCQ: hydroxychloroquine; IST: immunosuppressive therapy; MTX: methotrexate; PJP: *Pneumocystis jirovecii* pneumonia; RPILD: rapidly progressive interstitial lung disease; Tac: tacrolimus; TCZ: tocilizumab; TMP/SMZ: trimethoprim-sulfamethoxazole; TOF: tofacitinib. The bold text highlights significant values.

administration served as a protective factor against PJP development (adjusted OR=0.302, 95% CI of 0.116, 0.789) (Table II).

In multivariate analysis, all statistically significant factors were included in the final model (Table II). We found that after adjusting for confounding factors, older age (adjusted OR=1.045, 95% CI of 1.006, 1.086) and high cumulative dosage of GCs (adjusted OR=4.07, 95% CI of 1.336, 12.124) were independent risk factors for PJP occurrence in anti-MDA5+DM patients. Receiving prophylactic-dose TMP/SMZ was an independent protective factor (adjusted OR=0.212, 95% CI of 0.065, 0.689).

Predictors for PJP diagnosis in anti-MDA5+DM patients

Laboratory findings. Some blood cell count parameters were significantly lower in the PJP+ group than PJP- group. These differences were found in the count of neutrophils, lymphocytes, neutroplil lymphocyte ratio (NLR), B-cells, T-cells, $CD4^+$ T-cells, and CD8+ T-cells ($p<0.001$, $p=0.001$, $p<0.001$, $p=0.014$, $p=0.007$, $p=0.008$, $p=0.049$, Supplementary Table S1). There were significant amounts of patients in both groups that showed elevated levels of serum lactic acid dehydrogenase (LDH), ferritin (FET), and C-reactive protein (CRP). When compared with the PJP- group, the serum LDH ($p=0.02$), FET, ($p=0.013$) and CRP ($p=0.02$) levels were further increased in the PJP+ group. It should be noted that the concentration of serum brain natriuretic peptide in the PJP+ group was significantly higher than that in the PJP- group ($p=0.03$), suggesting that PJP may increase cardiac load.

Development of a predictive model for identifying PJP in anti-MDA5+ DM patients. In multivariate analysis, the count of neutrophils, $CD4^+$ T-cells, $CD8^+$ T-cells, CRP, LDH, and FET were included in the logistic regression analysis (Supplementary Table S2). Results showed that $CD4^+$ T-cells count (adjusted OR=0.001, 95% CI of 0, 0.177) and serum LDH level (adjusted OR=4.07, 95% CI of 1.001, 1.008) were independent indicators for PJP occurrence. Based on regression coefficients, we established a combination variable: combination factor = LDH-CD4*7.411/0.004. The combination factor was proved to be a good predictor for PJP occurrence with an area under the curve of 0.819 by ROC curve analysis (Fig. 2, Supplementary Table S3).

Possible prognostic indicators for anti-MDA5+ DM patients with PJP

In the PJP+ group, a total of 26 patients died within 30 days after PJP diagno-

Table II. Univariate and multivariate binary logistic regression analysis to identify risk factors for PJP occurrence in patients with anti-MDA5+DM.

Variables	Groups	Univariate analysis			Multivariate analysis		
		p-value	OR	95%CI	*p*-value	OR	95%CI
Age, years	-	**0.029**	1.039	1.004, 1.075	**0.023**	1.045	1.006, 1.086
Diabetes	-	**0.041**	2.381	1.035, 5.477	0.113	2.199	0.831, 5.818
Disease course of DM, months	>6 ≤6[a]	**0.039**	0.341	0.123, 0.946	0.083	0.352	0.108, 1.148
High cumulative dosage of GCs[c]	-	**0.034**	2.545	1.074, 6.032	**0.012**	4.07	1.366, 12.124
TMP/SMZ prophylaxis (prophylacic-dose)	-	**0.015**	0.302	0.116, 0.789	**0.01**	0.212	0.065, 0.689

[a]Data used as the control; [b]The average daily dose of GC used during 28 days before the diagnosis of PJP and expressed as the prednisone equivalent dose. [c]Prednisone used at least 30mg daily for at least 28 days or equivalent GCs within 3 months preceding PJP diagnosis.
Anti-MDA5+DM: anti-melanoma differentiation-associated gene 5 antibody-positive dermatomyositis; DM: dermatomyositis; GCs: glucocorticoids; PJP: *Pneumocystis jirovecii* pneumonia; TMP/SMZ: trimethoprim-sulfamethoxazole.
The bold text highlights significant values.

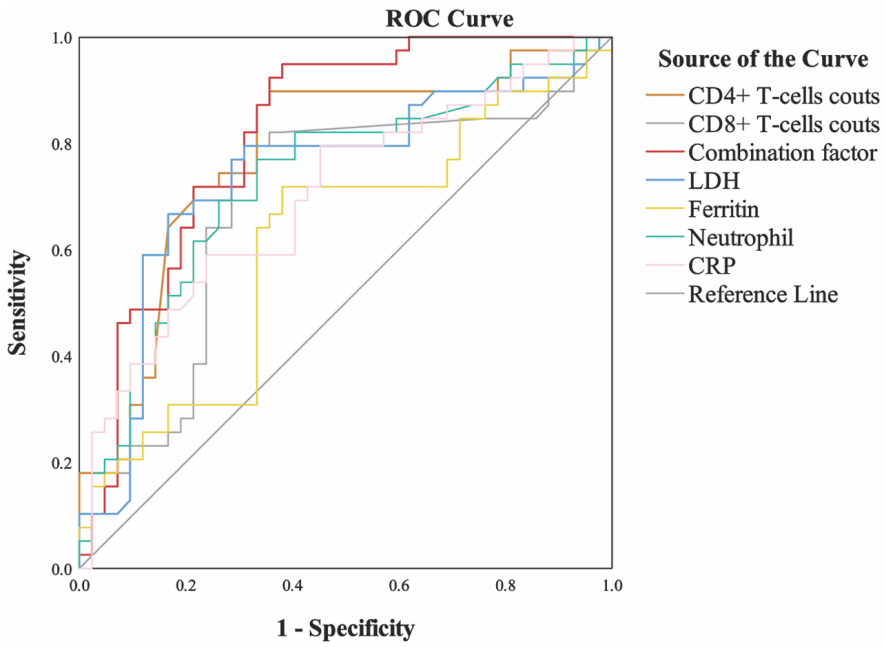

Fig. 2. Predictors for PJP diagnosis in anti-MDA5+DM patients.
Combination factor 1=LDH-CD4*7.411/0.004. Anti-MDA5+DM: anti-melanoma differentiation-associated gene 5 antibody-positive dermatomyositis; CRP: C-reactive protein; FET: ferritin; LDH: lactic acid dehydrogenase; N: neutrophil count; PJP: *Pneumocystis jirovecii* pneumonia.

sis, with a total mortality rate of 55.3% significantly higher than 30% in the PJP- group (p=0.008) (Table I). To explore possible prognostic factors, we carried out different analyses between survivors and non-survivors in PJP+ patients.

In the survival group, treated with GCs (p=0.019), calcineurin inhibitors (CNIs, p=0.044) were more prevalent and there was a trend of higher utilisation rate of biological or targeted synthesized DMARDs (p=0.07) (Table III). In contrast, moderate to severe acute respiratory distress syndrome (ARDS), mechanical ventilation, low oxygenation index, and extensive radiological ILD were more common in dead patients than those who survived (p=0.009, p=0.024, p<0.001, p=0.003, respectively). We also found that the levels of serum FET and LDH were higher in the death group (all p<0.001). In univariate logistic analysis, we identified short duration of DM (adjusted OR=0.725, 95% CI of 0.543, 0.968) and IST (adjusted OR=0.472, 95% CI of 0.262, 0.851), mechanical ventilation (adjusted OR=12.5, 95% CI of 1.444, 108.193), low oxygenation index (adjusted OR=0.986, 95% CI of 0.978, 0.994), moderate to severe ARDS (adjusted OR=17.85, 95% CI of 4.137, 77.018) and extensive radiological ILD (adjusted OR=1.039, 95% CI of 1.011, 1.068) as risk factors for death in PJP+ patients. We also identified GCs therapy (adjusted OR=0.158, 95% CI of 0.03, 0.832) as a protective factor by univariate analysis.

Discussion

In this study, anti-MDA5+DM patients with negative results of *pneumocystis jirovecii* were included as the control group. While this approach introduced some heterogeneity in controls, it enhanced the real-world clinical relevance of our findings. This study reveals a high morbidity and mortality of PJP in patients with anti-MDA5+DM. Older age and high cumulative dosage of GCs increase the risk of PJP. In anti-MDA5+DM, making a definitive diagnosis of PJP is difficult with unique routine test. A combination of elevated LDH and low $CD4^+$ T-cell count could be a well performed predictor for PJP occurrence. 30-day mortality rate of anti-MDA5+DM patients with PJP was as high as 55.3%, despite that 92.3% of these patients had been treated with therapeutic-dose TMP/SMZ or carpofungine. Patients with severe hypoxia and extensive radiological ILD may have worse outcomes.

GCs have been identified as a risk factor for PJP (16). In a large retrospective study in the general population and patients with AIRD, the daily dosage of GCs over 10mg was found to confer the highest risk for PJP (1). In our study, high cumulative dosage of GCs was the

Table III. Different analysis between survivors and non-survivors in the PJP group for possible prognostic factors.

Variables	Survivors (n=21)	Non-survivors (n=26)	*p*-value	Univariate analysis, OR (95%CI)
Age, mean ± SD, years	54.6±13.3	58.9±9.2	0.192	-
Male gender, n%	10 (47.6)	13 (50.0)	0.871	-
Diabetes, n%	6 (28.6)	14 (53.8)	0.081	-
Disease course of DM, median (IQR), months	4 (6)	2 (1)	**0.001**	0.725 (0.543, 0.968)
Clinical manifestations, n%				
Fever	16 (76.2)	18 (69.2)	0.596	-
Cough	13 (61.9)	15 (57.7)	0.626	-
White sputum	7 (33.3)	11 (42.3)	0.529	-
Moderate and severe ARDS (P/F ratio≤200)	4 (19)	21 (80.7)	**<0.001**	17.85 (4.137, 77.018)
Initial laboratory indicators and radiological findings				
LDH, median (IQR), IU/L	388 (206) [a]	530 (209) [b]	**0.024**	1.004 (1, 1.008)
FET, median (IQR), ng/ml	913 (411) [a]	1932 (1911) [b]	**0.009**	1.001 (1, 1.001)
BNP, median (IQR), pg/ml	371 (952)	354 (485)	0.63	-
Oxygenation index, median (IQR)	295.0 (219.5)	94.5 (100.7)	**<0.001**	0.986 (0.978, 0.994)
The extent of ILD, median (IQR)	25 (45) [c]	65 (45) [d]	**0.003**	1.039 (1.011, 1.068)
Treatment, three months before pathogen detection				
Duration of IST, median (IQR), days	58 (109) [e]	5 (41) [f]	**0.001**	0.472 (0.262, 0.851)
Steroid used, n %	19 (90.5) [e]	15 (57.7) [f]	**0.019**	0.158 (0.03, 0.832)
Daily dosage, median (IQR), mg/day [g]	35.0 (46.7) [e]	14.0 (58.5) [f]	0.404	-
Cumulative time at-risk, median (IQR), days [h]	38 (62)	17 (43)	0.218	-
High cumulative dosage, n % [i]	9 (42.9)	10 (38.5)	0.760	-
csDMARDs used	13 (50.0) [e]	8 (38.1) [f]	0.094	-
Tac, n%	6 (28.6) [e]	3 (11.5) [f]	0.158	-
CsA, n%	3 (14.3) [e]	1 (3.8) [f]	0.217	-
CTX, n%	5 (23.8) [e]	4 (15.4) [f]	0.506	-
CNIs, n%	9 (42.9) [e]	4 (15.4) [f]	**0.044**	0.254 (0.064, 1.004)
Combination of csDMARDs, n%	4 (19.0) [e]	1 (3.8) [f]	0.102	-
Bio/target DMARDs used, n% [j]	7 (33.3) [e]	3 (11.5) [f]	0.07	-
Treatment for PJP				
Therapeutic-dose TMP/SMZ, n%	18 (85.7)	22 (84.6)	0.916	-
Carpofungine, n%	7 (33.3)	9 (34.6)	0.927	-
TMP/SMZ or carpofungine, n%	19 (90.4)	24 (92.3)	0.823	-
Mechanical ventilation, n%	1 (4.8)	10 (38.5)	**0.007**	12.5 (1.444, 108.193)

[a]Data available for 15 patients; [b]Data available for 24 patients; [c]Data available for 18 patients; [d]Data available for 21 patients. [e]Data available for 21 patients; [f]Data available for 25 patients; [g]The average daily dose of GCs used during 28 days before the examination of *Pneumocystis jirovecii* and expressed as the prednisolone equivalent dose. [h]Days with prednisone used at least 20 mg/day or equivalent GCs within 3 months preceding PJP diagnosis; [i]Prednisone used at least 30mg daily for at least 28 days or equivalent GCs within 3 months preceding PJP diagnosis. [j]Bio/target DMARDs refer to tofacitinib and tocilizumab. ARDS: acute respiratory distress syndrome; Bio/target DMARDs: biological/targeted synthetic disease modifying anti-rheumatic drugs; BNP: brain natriuretic peptide; CNIs: calcineurin inhibitors; CsA: cyclosporine A; csDMARDs: conventional synthesized disease modifying anti-rheumatic drugs; CTX: cyclophosphamide; DM: dermatomyositis; FET: ferritin; GCs: glucocorticoids; ILD: interstitial lung disease; IST: immunosuppressive therapy; LDH: lactic acid dehydrogenase; P/F: ratio of partial pressure of O2 in arterial blood to fraction of inspired oxygen; PJP: *Pneumocystis jirovecii* pneumonia; RPILD: rapidly progressive interstitial lung disease; Tac: tacrolimus; TMP/SMZ: trimethoprim-sulfamethoxazole; Bold text highlights significant values.

critical risk driver for PJP occurrence, rather than average daily dose or duration at-risk. For prognosis, GCs therapy is a protective factor for survival in anti-MDA5+DM with PJP. The survival benefit of GCs lacked a clear dose-response relationship in our cohort. Because the differences in average daily dosage, cumulative time at-risk, or high cumulative dosage were not significant between survival and death patients. To better understand the role of GCs in anti-MDA5+DM patients with PJP, further study are needed. Patients with RPILD often receive aggressive regimens may partially explain why PJP was more common in patients with short courses. In addition, severe immune dysfunction, often characterised by higher disease activity, in the early stage of anti-MDA5+DM could also drive a higher susceptibility to PJP. This is consistent with previous finding of a predisposion to PJP infection in patients within two months after diagnosis of anti-MDA5+DM (7). Therefore, patients who have recently initiated immunosuppressive therapy need to be vigilant against PJP.

Lymphopenia, particularly reduced $CD8^+$ T-cells counts, has been widely reported as a diagnostic and prognostic marker for PJP (13, 17, 18). Our study, as well as previous studies in connective tissue disease, reported lower lymphocyte and $CD8^+$ T-cells count in patients with PJP (19). However, reduced lymphocyte and $CD8^+$ T-cells in anti-MDA5+DM patients are not appropriate as a predictor for PJP, as it may be due to the disease itself. In addition, we also found lymphocyte and $CD8^+$ T-cells count were not associated with the prognosis of PJP in anti-MDA5+DM patients.

TMP/SMZ is considered the first-line drug for both prevention and treatment of PJP (20, 21). Studies highlighted

the role of TMP/SMZ in reducing the incidence of PJP, but not the mortality rate (22). In our study, no significant difference between survivors and non-survivors receiving therapeutic-dose TMP/SMZ, aligning with prior studies, suggesting that TMP/SMZ did not improve PJP outcomes in anti-MDA5+ DM patients. Other studies have reported that pentamidin and atovaquone can be used as second-line therapy in patients who do not respond to TMP/SMZ (23). To precisely deliver prophylaxis treatment, we need to identify patients at high risk for PJP. Patients with PJP who do not respond well to TMP/SMZ should promptly consider alternative options.

This study has some limitations. First, as a single-center retrospective study, our study may be influenced by intrinsic bias. Second, co-infections were not further analysed in this study. Well-designed multicentre prospective studies are needed to further elaborate our findings.

Conclusions

PJP significantly increased the mortality of anti-MDA5+DM patients. Clinicians should identify high-risk patients early and initiate prophylactic-dose TMP/SMZ for PJP prophylaxis.

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References

1. HSU HC, CHANG YS, HOU TY *et al*.: Pneumocystis jirovecii pneumonia in autoimmune rheumatic diseases: a nationwide population-based study. *Clin Rheumatol* 2021; 40(9): 3755-63. https://doi:10.1007/s10067-021-05660-4
2. HE L, GE Y, LI S *et al*.: Clinical role of bronchoalveolar lavage in dermatomyositis-associated interstitial lung disease. *Rheumatology* (Oxford) 2021; 61(1): 345-54. https://doi:10.1093/rheumatology/keab586
3. SHIRAI T, MACHIYAMA T, SATO H, ISHII T, FUJII H: Intensive induction therapy combining tofacitinib, rituximab and plasma exchange in severe anti-melanoma differentiation-associatedprotein-5 antibody-positive dermatomyositis. *Clin Exp Rheumatol* 2023; 41(2): 291-300. https://doi:10.55563/clinexprheumatol/8kulbf
4. TINIAKOU E, MECOLI CA, KELLY W *et al*.: Anti-MDA5-positive dermatomyositis and remission in a single referral centre population. *Clin Exp Rheumatol* 2023 ;41(2): 309-15. https://doi:10.55563/clinexprheumatol/g4l70r
5. GE YP, SHU XM, HE LR, WANG GC, LU X: Infection is not rare in patients with idiopathic inflammatory myopathies. *Clin Exp Rheumatol* 2022; 40(2): 254-9. https://doi:10.55563/clinexprheumatol/yps7ai
6. REDONDO-BENITO A, CURRAN A, VILLAR-GOMEZ A *et al*.: Opportunistic infections in patients with idiopathic inflammatory myopathies. *Int J Rheum Dis* 2018; 21(2): 487-96. https://doi:10.1111/1756-185x.13255
7. HUANG L, FU Q, YE Y, LIN Y, YAN Q, CHEN S: High incidence and mortality of Pneumocystis jirovecii infection in anti-MDA5-antibody-positive dermatomyositis: experience from a single center. *Arthritis Res Ther* 2021; 23(1): 232. https://doi:10.1186/s13075-021-02606-8
8. BOHAN A, PETER JB: Polymyositis and dermatomyositis (first of two parts). *N Engl J Med* 1975; 292(7): 344-7. https://doi:10.1056/nejm197502132920706
9. LUNDBERG IE, TJÄRNLUND A, BOTTAI M *et al*.: 2017 European League Against Rheumatism/American College of Rheumatology classification criteria for adult and juvenile idiopathic inflammatory myopathies and their major subgroups. *Ann Rheum Dis* 2017; 76(12): 1955-64. https://doi:10.1136/annrheumdis-2017-211468
10. MAMMEN AL, ALLENBACH Y, STENZEL W, BENVENISTE O: 239th ENMC International Workshop: Classification of dermatomyositis, Amsterdam, the Netherlands, 14-16 December 2018. *Neuromuscul Disord* 2020; 30(1): 70-92. https://doi:10.1016/j.nmd.2019.10.005
11. SATO S, MURAKAMI A, KUWAJIMA A *et al*.: Clinical Utility of an Enzyme-Linked Immunosorbent Assay for Detecting Anti-Melanoma Differentiation-Associated Gene 5 Autoantibodies. *PloS one* 2016; 11(4): e0154285. https://doi:10.1371/journal.pone.0154285
12. LIU CJ, LEE TF, RUAN SY, YU CJ, CHIEN JY, HSUEH PR: Clinical characteristics, treatment outcomes, and prognostic factors of Pneumocystis pneumonia in non-HIV-infected patients. *Infect Drug Resist* 2019; 12: 1457-67. https://doi:10.2147/idr.S199761
13. TANG G, TONG S, YUAN X *et al*.: Using Routine Laboratory Markers and Immunological Indicators for Predicting Pneumocystis jiroveci Pneumonia in Immunocompromised Patients. *Front Immunol* 2021; 12: 652383. https://doi:10.3389/fimmu.2021.652383
14. TRAVIS WD, COSTABEL U, HANSELL DM *et al*.: An official American Thoracic Society/European Respiratory Society statement: Update of the international multidisciplinary classification of the idiopathic interstitial pneumonias. *Am J Respir Crit Care Med* 2013; 188(6): 733-48. https://doi:10.1164/rccm.201308-1483ST
15. WON HUH J, SOON KIM D, KEUN LEE C *et al*.: Two distinct clinical types of interstitial lung disease associated with polymyositis-dermatomyositis. *Respir Med* 2007; 101(8): 1761-9. https://doi:10.1016/j.rmed.2007.02.017
16. CHEW LC, MACEDA-GALANG LM, TAN YK, CHAKRABORTY B, THUMBOO J: Pneumocystis jirovecii pneumonia in patients with autoimmune disease on high-dose glucocorticoid. *J Clin Rheumatol* 2015; 21(2): 72-5. https://doi:10.1097/rhu.0000000000000215
17. JIN F, XIE J, WANG HL: Lymphocyte subset analysis to evaluate the prognosis of HIV-negative patients with pneumocystis pneumonia. *BMC Infect Dis* 2021; 21(1): 441. https://doi:10.1186/s12879-021-06124-5
18. LI Y, GHANNOUM M, DENG C *et al*.: Pneumocystis pneumonia in patients with inflammatory or autoimmune diseases: Usefulness of lymphocyte subtyping. *Int J Infect Dis* 2017; 57: 108-15. https://doi:10.1016/j.ijid.2017.02.010
19. LI J, HUANG XM, FANG WG, ZENG XJ: Pneumocystis carinii pneumonia in patients with connective tissue disease. *J Clin Rheumatol* 2006; 12(3): 114-7. https://doi:10.1097/01.rhu.0000221794.24431.36
20. PARK JW, CURTIS JR, MOON J, SONG YW, KIM S, LEE EB: Prophylactic effect of trimethoprim-sulfamethoxazole for pneumocystis pneumonia in patients with rheumatic diseases exposed to prolonged high-dose glucocorticoids. *Ann Rheum Dis* 2018; 77(5): 644-9. https://doi:10.1136/annrheumdis-2017-211796
21. SALZER HJF, SCHÄFER G, HOENIGL M *et al*.: Clinical, Diagnostic, and Treatment Disparities between HIV-Infected and Non-HIV-Infected Immunocompromised Patients with Pneumocystis jirovecii Pneumonia. *Respiration* 2018; 96(1): 52-65. https://doi:10.1159/000487713
22. LI R, TANG Z, LIU F, YANG M: Efficacy and safety of trimethoprim-sulfamethoxazole for the prevention of pneumocystis pneumonia in human immunodeficiency virus-negative immunodeficient patients: A systematic review and meta-analysis. *PloS One* 2021; 16(3): e0248524. https://doi:10.1371/journal.pone.0248524
23. KITAZAWA T, SEO K, YOSHINO Y *et al*.: Efficacies of atovaquone, pentamidine, and trimethoprim/sulfamethoxazole for the prevention of Pneumocystis jirovecii pneumonia in patients with connective tissue diseases. *J Infect Chemother* 2019; 25(5): 351-4. https://doi:10.1016/j.jiac.2019.01.005