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Cluster analysis reveals three clinical phenotypes of pulmonary artery hypertension associated with connective tissue diseases: insights into inflammation and immunity

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Abstract

Background Inflammation and immune mechanisms play a crucial role in connective tissue disease-associated pulmonary arterial hypertension (CTD-PAH), though they remain inadequately understood. This study aimed to identify specific clinical phenotypes in CTD-PAH using inflammatory and immune markers through hierarchical cluster analysis.

Methods We conducted a single-center, retrospective cohort study of CTD-PAH patients from 2009 to 2024. Clinical variables, including neutrophil lymphocyte ratio (NLR), erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), and complement C3 and C4, were analyzed to form clusters based on baseline characteristics, clinical outcomes, and treatment goals.

Results Among 184 patients (95.1% female; median age 40.42 years), three distinct clusters were identified: Cluster 1 (vasculopathic phenotype) exhibited lower inflammatory activity but worse hemodynamic outcomes; Cluster 2 (vasculitic phenotype) had higher inflammatory activity with favorable hemodynamics; Cluster 3 (mixed phenotype) showed active inflammation and poor hemodynamic status. Most vasculitic patients were classified as systemic lupus erythematosus-associated PAH (SLE-PAH), which had a shorter course and higher prevalence of autoantibodies. The vasculopathic and mixed phenotypes were common in scleroderma-related PAH (SSc-PAH), undifferentiated CTD-

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related PAH (UCTD-PAH), and mixed CTD- related PAH (MCTD-PAH), associated with poorer treatment outcomes and survival rate.

Conclusion Distinct clinical phenotypes in CTD-PAH correlate with inflammatory activity and hemodynamic status, influencing treatment responses and prognosis.

Clinical Perspectives

- Inflammation and immune mechanisms are essential for the development of CTD-PAH.
- Three distinct phenotypes in CTD-PAH were identified through cluster analysis.
- Distinct phenotypes correlate with inflammatory and hemodynamic status, influencing treatment responses and prognosis.
- Identifying disease phenotypes might improve the management algorithm for CTD-PAH.

Keywords Connective tissue disease, Pulmonary arterial hypertension, Inflammation, Immunity, Mechanisms, Prognosis

Introduction

Pulmonary arterial hypertension (PAH) is a severe cardiopulmonary disorder characterized by progressive vascular remodeling [1]. Its pathogenesis is complex and not yet fully understood. Recent studies highlight the roles of inflammation and immune responses in PAH development, indicating that it is not solely driven by vasoconstriction but also involves immune dysfunction [2, 3].

Connective tissue diseases (CTD) are often associated with chronic inflammation, featuring persistent immune cell infiltration and elevated pro-inflammatory cytokines and chemokines [4–6]. There is growing evidence of inflammatory and immune cells presence in the lungs of PAH patients, particularly in those with connective tissue diseases (CTD-PAH) [3, 5]. Cytokines such as interleukin-1β (IL-1β), interleukin-6 (IL-6), and tumor necrosis factor-α (TNF-α), along with increased autoantibodies, have been linked to disease severity and mortality [2–5]. While systemic sclerosis (SSc) is the most common CTD-PAH in Western countries, systemic lupus erythematosus (SLE) is the leading cause in China. In SLE, immune-mediated mechanisms contribute to PAH through endothelial and smooth muscle cell proliferation, fibrinoid necrosis from vasculitis, and deposition of immunoglobulins in pulmonary vessels [7]. Conversely, SSc-PAH typically shows non-inflammatory vascular remodeling [7–9]. Interestingly, some SLE-PAH patients exhibit vasculopathy similar to SSc-PAH, suggesting that immune infiltration may influence the pathogenesis and prognosis of different CTD-PAH subtypes [7–11].

Despite progress, the inflammatory and immunological mechanisms in CTD-PAH remain inadequately elucidated. While genetic and molecular phenotyping offer insights, current knowledge does not sufficiently identify disease subtypes based on clinical variables. Markers such as erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), complements level, and changes in neutrophil and lymphocyte counts provide key indicators of inflammation and immune dysfunction [12–14]. Clustering analysis can effectively identify subtypes in complex diseases, serving as a hypothesis-generating tool [15, 16].

This study aims to identify specific clinical phenotypes in CTD-PAH using common clinical markers of inflammation and immunity through hierarchical cluster analysis, and to explore associations between these phenotypes and patient outcomes.

Materials and methods

Study design

This retrospective analysis examined data from consecutively diagnosed patients with CTD-PAH admitted to the First Affiliated Hospital of Nanjing Medical University between January 2009 and June 2024 (NCT05980728). The study received approval from the Medical Ethics Committee of the First Affiliated Hospital of Nanjing Medical University (approval number 2018-SR-333) and adhered to the principles outlined in the Declaration of Helsinki. Informed consent was obtained from all prospective participants.

Patients

Inclusion criteria: The study included patients aged over 18 years who were diagnosed with both a CTD and PAH. The diagnosis of CTDs was based on specific criteria: SLE according to the 2019 EULAR/ACR criteria [17], primary Sjogren's Syndrome (pSS) as defined by the 2016 ACR criteria [18], Systemic Sclerosis (SSc) according to the 2013 ACR/EULAR criteria [19], Rheumatoid Arthritis (RA) based on the 2010 ACR/EULAR criteria [20], and Mixed Connective Tissue Disease (MCTD) as per the Sharp criteria [21]. Patients who had clinical and serological manifestations suggestive of systemic autoimmune diseases but did not fulfil the classification criteria for CTD were defined as undifferentiated CTD (UCTD). The diagnosis of PAH was established by criteria including a mean pulmonary arterial pressure (mPAP) > 20 mmHg, pulmonary arterial wedge pressure (PAWP) ≤ 15 mmHg,

and pulmonary vascular resistance (PVR) > 2 Wood units at rest determined via right heart catheterization (RHC) [11].

Exclusion criteria: (i) PAH co-induced by other factors; (ii) Patients with evidence of restrictive or obstructive lung diseases, such as significant chronic obstructive pulmonary disease, restrictive ventilatory defects, moderate to severe obstructive sleep apnea, and a diagnosis of moderate or severe interstitial lung disease by clinical assessment and/or findings on chest X-ray or computed tomography; (iii) Patients with clear indications of serious infection; (iv) Patients who were lost to follow-up.

Data collection

Baseline data were defined as those collected at the time of the first confirmed diagnosis of PAH via RHC. A standardized case report form was developed to systematically gather baseline and follow-up data, encompassing demographic details, clinical characteristics (including underlying CTDs, course of PAH, World Health Organization functional class (WHO-FC), and 6-minute walk distance (6MWD)), laboratory findings (pertinent to both CTD and PAH evaluation), risk stratification, echocardiographic parameters and RHC data. Baseline therapeutic regimens were documented, covering supportive care, management of the underlying CTD, and targeted therapy for PAH. Follow-up data included survival outcomes, clinical worsening, treatment goals, and risk stratification over the monitoring period.

Laboratory analyses

Laboratory analyses were performed on baseline samples. We selected the neutrophil-to-lymphocyte ratio (NLR) as an inflammatory marker because numerous studies suggest it reliably reflects the body's immune balance and predicts prognosis in PAH [22, 23]. Although a definitive cutoff value remains elusive, the accessibility of NLR via routine complete blood counts. NLR was calculated by dividing the absolute neutrophil count by the absolute lymphocyte count, as obtained from a Mindray fully automated hematology analyzer. C-reactive protein (CRP) was measured by high-sensitivity CRP nephelometry, with a range of 0.1 to 6 mg/L. Erythrocyte sedimentation rate (ESR) was determined using the Capillary Photometry Method. Reference ranges for ESR are age- and sex-specific, approximately as follows: (a) Men: Under 50 years: 0–21 mm/h; Over 50 years: 0–43 mm/h; (b) Women: Under 50 years: 0–26 mm/h; Over 50 years: 0–38 mm/h. Plasma C3 and C4 levels were quantified via immunoturbidimetry (normal range: C3, 0.7–1.4 g/L; C4, 0.1–0.4 g/L).

Follow-up and study assessments

Follow-up data were collected through a combination of medical record reviews and telephone consultations, spanning from the date of PAH diagnosis to August 30, 2024.

The primary end point was the time from baseline to first adjudicated clinical worsening. Clinical worsening was defined based on criteria established in the AMBITION trial [24] as (i) all-cause mortality, (ii) lung or heart/lung transplant, (iii) hospitalization of PAH deterioration, (iv) unsatisfactory long-term clinical response (6MWD decreased ≥ 15% from baseline or WHO-FC sustain III/IV symptoms assessed at two clinic visits separated by 6 months after adequate treatment with PAH targeted drugs), or (v) add-on treatment with parenteral prostacyclin analogues. The time of clinical worsening was computed as the period in months from the initial PAH diagnosis to either the conclusion of the follow-up period or the occurrence of clinical worsening.

The secondary endpoint focused on achieving treat-to-target status within the first-year post-diagnosis, as outlined in the 2022 ESC/ERS Guidelines [11]. Treat-to-target were characterized by maintaining a low-risk status: (i) WHO-FC I or II; (ii) 6MWD > 440 m; and (iii) B-type natriuretic peptide (BNP) levels < 50ng/L or N-terminal pro-B-type natriuretic peptide (NT-pro BNP) levels < 300ng/L.

Statistical analysis

The statistical analyses were conducted utilizing SPSS version 26.0 (IBM Corp, Armonk, NY, USA), GraphPad Prism 9.0 (GraphPad Software, San Diego, USA), and R statistical software v4.3.2 (R Foundation for Statistical Computing, Vienna, Austria). Prior to hierarchical clustering of the selected inflammatory and immune variables (NLR, ESR, CRP, C3, and C4), missing data (< 20%) were imputed using multiple imputation by chained equations (package 'mice') to generate 5 imputed datasets. Two optimal datasets were selected as training sets and validation sets respectively for sensitivity analysis. Redundant metrics were then eliminated based on a correlation matrix, retaining the variable with the correlations < 0.8. Agglomerative hierarchical clustering was performed using the *hclust* function, with the dissimilarity matrix calculated using Euclidean distance and clusters joined using Ward's method. The optimal number of clusters was determined using the *NBclust* function/package, which selects the optimal number based on 27 clustering criteria. All clustering analyses were conducted blinded to imaging data, hemodynamic parameters, and outcomes.

Normality was assessed using the Kolmogorov-Smirnov test. Continuous variables were presented as mean ± standard deviation (SD) or as medians with interquartile

ranges, while categorical variables were expressed as absolute and relative frequencies (percentages). Group comparisons were performed using Student's t-test or the Wilcoxon test for two groups, and the one-way analysis of variance or the Kruskal–Wallis test for more than two groups. Post-hoc pairwise comparisons were conducted using the least significant difference-t test (LSD-t) or the Wilcoxon rank sum test. Proportional comparisons were assessed using Fisher's exact test with pairwise multiple tests. Differences in outcomes between groups were evaluated using Kaplan-Meier plots with omnibus and pairwise Log-Rank tests. The significance for statistical tests were set at a = 0.05.

Results

Characteristics of the whole cohort

In this retrospective analysis, 184 patients who met the specified inclusion and exclusion criteria were included (refer to Figure S1). The patient cohort was predominantly female, with nearly 95.1%. Among our patients with CTD-PAH, the most common underlying CTD subtypes were SLE at 45.7%, followed by pSS at 29.3%, SSc at 9.2%, UCTD at 6.5%, MCTD at 6.0%, and Other CTDs at 3.3%. The median age of the study participants was 40.42 years, and the median duration of PAH was 9.05 months, with the majority (93.5%) falling into WHO-FC II-III. Within the population, 48.4% tested positive for anti-Ro52 antibody, 59.5% for anti-Ro60 antibody, 40.1% for anti-U1 small nuclear ribonucleoprotein (anti-U1-snRNP) antibody, 23.2% for anti-double-stranded DNA (anti-dsDNA) antibody, 19.1% for anti-Smith (anti-Sm) antibody and 13.4% for anti-centromere antibody (see Table S1).

Hierarchical cluster analysis

The role of inflammation and immunity in the pathophysiology of PAH, particularly in the context of CTD-PAH, has been increasingly recognized in recent decades. In this study, we focused on five clinically relevant and accessible indicators - NLR, ESR, CRP, C3, and C4. Following a collinearity test, we found no significant correlations ($r < 0.8$) among the selected indicators. Utilizing cluster validity indices derived from the clinical data, we determined that the optimal number of clusters was three. The resulting dendrogram and heatmap, illustrating the division of patients into these distinct clusters, are presented in Figure S2.

Characteristics of inflammation and immunity across the identified clusters are as follows:

Cluster 1 ($n = 92$), the largest cluster, exhibited low activity in inflammatory and immune markers. Levels of ESR ($p < 0.001$ vs. Clusters 2 and 3) and CRP ($p < 0.05$ vs. Clusters 2 and 3) were notably lower compared to the other clusters, while levels of complement C3 ($p < 0.001$ vs. Clusters 2 and 3) and C4 ($p < 0.001$ vs. Cluster 2) were higher.

Cluster 2 ($n = 63$) demonstrated high activity in inflammatory and immune markers. Levels of ESR ($p < 0.001$ vs. Cluster 1) were elevated in comparison. Conversely, complement C3 ($p < 0.01$ vs. Clusters 1 and 3), and C4 ($p < 0.001$ vs. Clusters 1 and 3) levels were lower.

Cluster 3 ($n = 29$), the smallest cluster with moderately elevated activity in inflammatory and immune markers, displayed elevated levels of NLR ($p < 0.001$ vs. Clusters 1 and 2), CRP ($p < 0.001$ vs. Clusters 1 and 2), and ESR ($p < 0.001$ vs. Cluster 1). Notably, while levels of complement C3 ($p < 0.05$) and C4 ($p < 0.05$) were higher than in Cluster 2 (refer to Table 1).

Hemodynamic and echocardiographic characteristics of PAH in clusters

Cluster 1 and 3 exhibited poorer hemodynamic characteristics compared to Cluster 2 in our analysis of the three clusters. Specifically, Cluster 2 displayed more favorable hemodynamic profiles, characterized by lower mPAP, PVR, and higher cardiac index (CI) ($P < 0.05$).

Moreover, within Cluster 1, the tricuspid annular plane systolic excursion/pulmonary artery systolic pressure (TAPSE/PASP) ratio, a key echocardiographic parameter indicative of right ventricle-pulmonary artery (RV-PA) coupling, was notably lower than Clusters 2 and 3 ($P < 0.05$, as shown in Table 1; Fig. 1).

CTD characteristics of clusters

The distribution of different subtypes of CTD varied across the clusters in our analysis. Specifically, Cluster 2 showed a significantly higher proportion of SLE and a lower proportion of SSc and UCTD compared to Clusters 1 and 3 ($p < 0.05$). However, there was no statistically significant difference in the distribution of pSS, MCTD, and other CTDs among the clusters (Table 1). Furthermore, while there was no statistically significant difference in the use of immunosuppressants among the clusters, a higher proportion of patients in Cluster 2 and Cluster 3 received medium to high doses of glucocorticoids (defined as ≥ 0.5 mg/kg/day of prednisone or equivalent) compared to the Cluster 1 ($p < 0.05$, as shown in Table S2).

The differences in the antibody spectrum between the clusters were primarily driven by the higher positive rates of anti-Ro52, anti-U1-snRNP and SLE related specific antibodies (anti-dsDNA and anti-Sm antibodies) in Cluster 2 compared to Cluster 1 ($p < 0.05$). Among the three clusters, the positive rate of anti-Ro60 antibody was highest in Cluster 2 ($p < 0.05$, refer to Table 1; Fig. 2).

Table 1 Characteristics and differences of the 3 clusters

	Cluster 1 *N* = 92	Cluster 2 *N* = 63	Cluster 3 *N* = 29
Variables			
Age, years	40.39(31.06–53.66)	39.44(31.24–53.81)	46.19(33.64–59.13)
Female, n (%)	86(93.5)	62(98.4)	27(93.1)
CTD characteristics			
Course of CTD, months (Time from diagnosis of CTD to diagnosis of PAH)	12.53(0.03–83.43)	0.50(0.03–125.20)	52.50(0.10-123.75)
Protopathic CTDs			
SLE, n (%)	34(37)	40(63.5) **	10(34.5) ##
pSS, n (%)	27(29.3)	20(31.7)	7(24.1)
SSc, n (%)	11(12.0)	1(1.6) *	5(17.2) ##
MCTD, n (%)	5(5.4)	2(3.2)	4(13.8)
UCTD, n (%)	10(10.9)	0(0) **	2(6.9) #
Others, n (%)	5(5.4)	0(0)	1(3.4)
NLR	2.06(1.57–2.90) $$$	2.52(1.48–3.75)	8.00(5.29–10.97) ###
Monocyte, 10^9/L	0.46(0.34–0.62)	0.32(0.20–0.53) **	0.45(0.32–0.70)
eGFR, mL/min/1.73 m^2	104.54 ± 29.78	116.89 ± 42.04	100.41 ± 43.73
ESR, mm/h	10.50(4.25-22.00) $$$	52.00(26.00–86.00) ***	53.00(39.00–99.00)
CRP, mg/L	3.22(2.53–4.53) $$$	5.14(2.47-9.00) *	24.70(10.45–64.25) ###
IgG, g/L	11.70(9.85–15.41) $	22.30(15.00-33.10) ***	17.50(10.58–23.05) ##
C3, g/L	0.91 ± 0.19 $$$	0.59 ± 0.22 ***	0.73 ± 0.22 ##
C4, g/L	0.19 ± 0.06	0.11 ± 0.04 ***	0.17 ± 0.09 ###
Anti-Ro52+, n (%)	28(35.9)	38(64.4) ***	11(50)
Anti-Ro60+, n (%)	37(48.7)	43(78.2) ***	11(50) #
Anti-U1-snRNP+, n (%)	19(24.7) $$	31(52.5) ***	13(61.9)
Anti-dsDNA+, n (%)	11(15.1)	18(30.0) *	7(31.8)
Anti-Sm+, n (%)	8(10.4)	17(28.8) **	5(23.8)
Anti-centromere+, n (%)	12(15.6)	6(10.2)	3(14.3)
PAH characteristics			
Course of PAH, months (Symptom-to-diagnosis time for PAH)	14.52(3.44–40.26)	6.10(1.57–25.40)	4.37(1.49–33.54)
6MWD, m	407.45 ± 128.10	436.33 ± 109.97	401.80 ± 145.24
WHO-FC(I/II/III/IV), n	4/42/44/2	1/36/24/1	1/11/15/2
NT-pro BNP, pg/mL	844.10(231.00-2923.00)	480.75(179.93-1181.75)	1449.00(370.50–2782.00)
2018WSPH risk stratification			
Low/intermediate/ high risk, n	33/34/25	24/30/8	6/18/5
Right cardiac catheterization parameters			
Heart rate, beats/min	83.00(75.25-92.00)	81.00(76.00-95.75)	90.00(82.00-100.00)
mPAP, mmHg	44.00(37.00-51.75)	37.00(29.00–49.00) **	39.00(32.50–52.50)
PVR, Wood	8.78(6.02–13.55) $	5.36(3.86–9.16) ***	7.20(4.60–9.57)
RAP, mmHg	5.00(3.00–9.00)	5.00(3.00–7.00)	6.00(2.50-8.00)
CI, L/min/m^2	2.70(2.03–3.11)	3.20(2.75–3.90) ***	2.79(2.10–3.32) #
Two-dimensional echocardiography parameters			
RADI, mm/m^2	27.30 ± 4.61	26.40 ± 4.65	25.08 ± 4.66
RVDDI, mm/m^2	27.69 ± 4.40	26.79 ± 4.54	25.79 ± 5.21
TAPSE/PASP, mm/mmHg	0.21(0.16–0.28) $	0.27(0.18–0.34) *	0.30(0.19–0.36)
Pericardial effusion, n (%)	33(35.9)	28(45.2)	13(44.8)

Comparison between Cluster 1 and Cluster 2: *$P<0.05$, **$P<0.01$, ***$P<0.001$; Comparison between Cluster 2 and Cluster 3: #$P<0.05$, ##$P<0.01$, ###$P<0.001$; Comparison between Cluster 3 and Cluster 1: $$P<0.05$, $$$P<0.01$, $$$$P<0.001$. CTD: connective tissue disease, SLE: systemic lupus erythematosus, pSS: primary Sjogren's syndrome, SSc: systematic sclerosis, MCTD: mixed CTD, UCTD: undifferentiated CTD, RA: rheumatoid arthritis, NLR: neutrophil/lymphocyte ratio, Myc: monocyte count, eGFR: estimated glomerular filtration rate, ESR: erythrocyte sedimentation rate, CRP: C-reactive protein, IgG: immunoglobulin G, C3: complement C3, C4: complement C4, Anti-Ro52: antigen Ro52 antibodies, Anti-Ro60: antigen Ro60 antibodies, Anti-U1-snRNP: antigen U1 small nuclear ribonucleoprotein antibodies, Anti-dsDNA: anti-double-stranded DNA antibodies, Anti-Sm: anti-Smith antibodies, PAH: pulmonary arterial hypertension, 6MWD: 6-minute walking distance, WHO-FC: WHO functional class, NT-pro BNP: N-terminal pro-B-type natriuretic peptide, mPAP: mean pulmonary artery pressure, PVR: pulmonary vascular resistance, RAP: right atrium pressure, CI: cardiac index, RADI: right atrial end-systolic diameter index, RVDDI: right ventricular end-diastolic basal dimension index, TAPSE/PASP: tricuspid annular plane systolic excursion/pulmonary artery systolic pressure

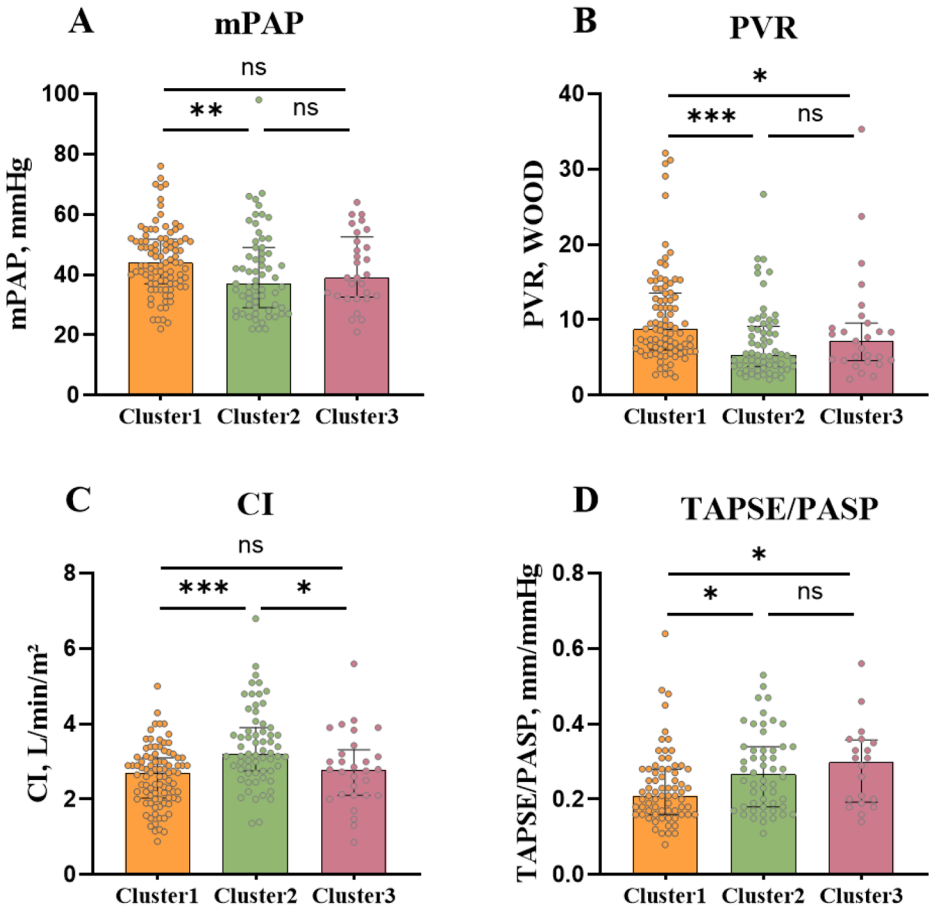

Fig. 1 Hemodynamic and echocardiographic characteristics of PAH in clusters. Scatterplots showing the differences in clusters at baseline for (**A**) mean pulmonary arterial pressure (mPAP); (**B**) pulmonary vascular resistance (PVR); (**C**) cardiac index (CI); and (**D**) tricuspid annular plane systolic excursion/pulmonary artery systolic pressure ratio (TAPSE/PASP). For scatterplots, each point represents an individual patient, lines show the median, and error bars show the interquartile ranges (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$)

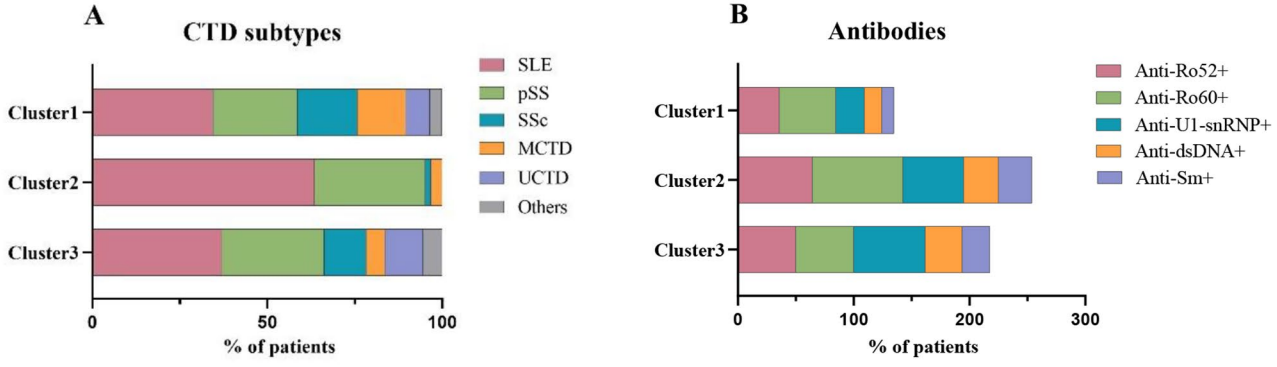

Fig. 2 Differences in CTD subtypes (**A**) and autoantibodies features (**B**) among clusters in patients with CTD-PAH. Cluster 2 showed a significantly higher proportion of SLE and lower proportion of SSc and UCTD compared to Clusters 1 and 3 ($P < 0.05$). A higher prevalence of Anti-Ro52+, Anti-Ro60+, Anti-U1 snRNP+, Anti-dsDNA + and Anti-Sm + antibodies was observed in the Cluster 2 ($P < 0.05$)

Clinical phenotype associations with clusters

In our results, Cluster 1 exhibited lower activity in inflammatory and immune markers but displayed a poorer hemodynamic profile in PAH This was characterized by normal range of C3, C4, ESR, and CRP, along with elevated PVR and mPAP, as well as decreased CI and TAPSE/PASP. This cluster predominantly represents the vasculopathic phenotype.

On the other hand, Cluster 2 demonstrated higher levels of inflammatory and immune activity, as evidenced by significantly elevated ESR, along with decreased levels of C3 and C4. Despite this, the hemodynamic characteristics of PAH in Cluster 2 were more favorable, with lower PVR and mPAP, and higher CI and TAPSE/PASP. This cluster is primarily associated with the vasculitic phenotype.

Cluster 3 displayed active inflammatory and immune markers along with worse hemodynamic parameters. Markers such as NLR, ESR, and CRP were elevated, while PVR was higher and CI was lower. This cluster represents a mixed phenotype characterized by features of both the vasculopathic and vasculitic phenotypes (refer to Table 1).

Associations between clinical phenotypes and survival outcomes

Treat-to-target achievement was defined as either maintaining or improving to a low-risk stratum according to the 2022 ESC/ERS Guidelines. Notably, patients in the vasculitic phenotype were more likely to achieve treat-to-target status compared to those in the vasculopathic phenotype and the mixed phenotype, with 1-year treat-to-target rates of 71.4% (vasculitic phenotype), 39.1% (vasculopathic phenotype), and 37.9% (mixed phenotype), respectively ($P<0.001$, see Fig. 3A). The results remained significant after adjusting for therapy of PAH and CTD ($P<0.001$, Fig. 3B). Similar results were observed in sensitivity analysis with another imputed dataset (Figure S3).

During the median follow-up period of 28 months, a total of 22 patients (20.0%) died and 52 patients (28.3%) experienced clinical worsening. The Kaplan-Meier curves for the three groups identified by hierarchical cluster analysis of selected variables are presented in Fig. 4. The survival rate of the vasculitic phenotype was found to be higher than that of the vasculopathic phenotype and the mixed phenotype (Log-rank $P=0.004$) based on pairwise Log-Rank testing (Fig. 4A). While the mixed subgroup exhibited the lowest survival rate compared to the vasculopathic subgroup (Log-rank $P=0.039$). The 1-, 3-, and 5-year event-free survival rates of vasculitic phenotype were reported as 94.0%, 86.4%, and 78.9%, respectively. Similar results were observed in Kaplan-Meier survival curve analysis using clinical worsening as the clinical outcome (Fig. 4B), as well as sensitivity analysis with another imputed dataset (Figure S4).

Discussion

The pathological mechanisms underlying CTD and PAH remain complex and not fully understood. Recent studies emphasize the role of inflammation and immune responses in the onset and progression of CTD-PAH [3–5]. However, the clinical relevance of inflammatory and immune indicators in guiding disease prognosis in CTD-PAH is still ambiguous. In this study, we focused on common clinical markers of inflammation and immunity (NLR, ESR, CRP, C3, and C4) through cluster analysis to assess their potential value in relation to specific PAH characteristics in CTD-PAH patients.

Our findings confirm the heterogeneous nature of CTD-PAH patients. Hierarchical cluster analysis revealed three distinct clinical phenotypes: (i) the vasculopathic phenotype (Cluster 1) showed low inflammatory and immune marker activity but poorer hemodynamic profiles; (ii) the vasculitic phenotype (Cluster 2) exhibited elevated inflammatory activity and better hemodynamics; (iii) the mixed phenotype (Cluster 3) displayed characteristics of both vasculopathic and vasculitic subtypes, with active inflammatory markers and worse

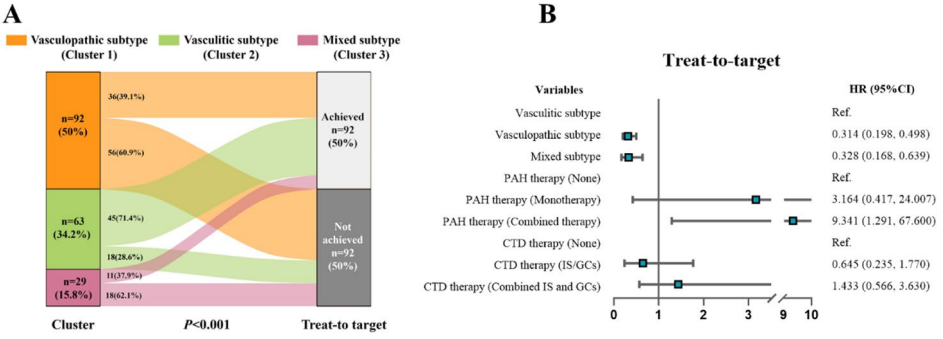

Fig. 3 The cumulative rate of treat-to-target within the first year between 3 clusters. Patients in Cluster 2 were more likely to achieve treat-to-target status compared to those in Cluster 1 and 3 ($P<0.001$). Association between 3 clusters and treat-to-target after adjusted for therapy of PAH and CTD ($P<0.001$)

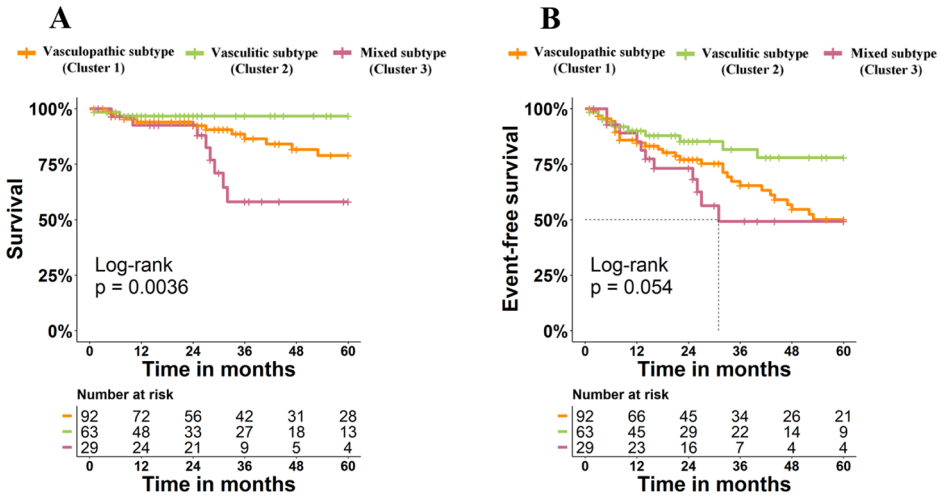

Fig. 4 Kaplan–Meier survival curves of CTD-PAH patients stratified by the three subtypes. (**A**) The survival rate of the mixed phenotype and the vasculopathic phenotype was found to be poorer than that of the vasculitic phenotype based on pairwise Log-Rank testing (log-rank $P=0.0036$). (**B**) The event-free survival rate of the mixed phenotype and the vasculopathic phenotype was found to be poorer than that of the vasculitic phenotype based on pairwise Log-Rank testing (log-rank $P=0.054$)

hemodynamic parameters. Importantly, patients with the vasculitic phenotype had significantly higher survival rates and were more likely to achieve treat-to-target status compared to those in the other groups.

Cluster 1, characterized by normal inflammatory markers but severe hemodynamic abnormalities (elevated PVR and mPAP, reduced CI), encompassed various CTD subtypes, with SLE being most prevalent (37.0%), followed by pSS (29.3%). This indicates that multiple CTD subtypes can present as a vasculopathic phenotype. Prior research by Junyan Qian et al. identified vasculitic and vasculopathic phenotypes in SLE-PAH, with the vasculitic subgroup showing a better prognosis [25]. Our findings align with this, revealing a poorer prognosis for the vasculopathic phenotype. Elevated PVR reflects ongoing vascular remodeling, influencing PAH prognosis [1]. With increasing PVR, the right ventricle (RV) adapts to the chronic elevation of afterload through compensatory hypertrophy, eventually leading to RV dilatation and dysfunction, transitioning from a coupled RV-PA state to uncoupling [26]. The potential for concomitant subclinical primary myocardial involvement, like microvascular disease as noted by Hsu S et al., could exacerbate RV maladaptive remodeling in SSc-PAH [27]. Patients with the vasculopathic phenotype had poorer hemodynamic performance, suggesting severe pulmonary vascular remodeling, and a lower TAPSE/PASP ratio indicated maladaptive RV remodeling. Despite modest inflammatory indices, these patients progressed to uncoupling between the RV and pulmonary artery, contributing to poor treatment responses and outcomes, underscoring the need for attention to these characteristics across CTD-PAH subtypes.

In Cluster 2, patients showed elevated inflammatory markers, including increased ESR and IgG levels, and decreased complement C3 and C4 levels, typical of a vasculitic phenotype. This phenotype was predominant in SLE-PAH (63.5%) and pSS-PAH (31.7%), consistent with previous reports of plexogenic lesions and fibrinoid vasculitis in SLE-PAH, but rare in SSc-PAH and MCTD-PAH [3, 7–9]. Notably, UCTD-PAH patients did not present vasculitic phenotypes, warranting further investigation with larger samples. The vasculitic phenotype exhibited a higher prevalence of autoantibodies, including anti-dsDNA, anti-Sm, anti-Ro52, anti-Ro60, and anti-U1-snRNP, known to enhance proinflammatory signals via immune complex formation [28–30], and previously implicated as PAH risk factors in CTD [31–33]. Interestingly, we found that higher positivity of anti-dsDNA and anti-Sm antibodies in the vasculitic phenotype versus the vasculopathic phenotype. Anti-dsDNA and anti-Sm antibodies have the potential to contribute significantly to vascular inflammation in SLE-PAH. Mechanisms such as immune complex deposition, complement activation, NETosis, and downstream cellular signaling could collectively contribute to the development of fibrinoid vasculitis and plexiform lesions characteristic of this condition [34]. Despite this heightened inflammatory state and autoantibody profile, patients in this cluster demonstrated favorable hemodynamics and survival, consistent with Sobanski V et al.'s findings on anti-U1-snRNP positivity [35]. The precise role of these and other autoantibodies in CTD-PAH prognosis remains to be fully elucidated.

Cluster 3, with only 29 patients (15.8%), displayed heightened inflammatory states and severe hemodynamic

abnormalities. This mixed phenotype exhibited both vasculopathic and vasculitic features, leading to the worst prognosis under the double strike. Although all CTD-PAH subtypes can present mixed phenotypes, it was particularly common in SSc-PAH and MCTD-PAH. Previous studies suggest these subtypes have poorer prognoses [36–37]. The presence of mixed phenotypes, especially in elderly patients, may indicate a convergence of multiple underlying pathophysiological mechanisms, contributing to the poor prognosis of these CTD-PAH subtypes.

In our cohort, 96.7% of patients received timely PAH-targeted therapy, and those treated with multiple agents achieved treatment goals swiftly within a year. A majority (91.8%) initially received CTD therapies. Notably, vasculitic and mixed phenotype patients tended to use higher glucocorticoid doses. Although initial treatments varied by phenotypes, they independently influenced PAH treatment success within a year, highlighting the need for more robust evidence linking phenotypes to optimal treatments and the necessity for larger clinical trials.

Limitations of our study include its retrospective, single-center nature, which may limit generalizability to broader CTD-PAH or PAH populations. The observational design precludes definitive conclusions about steroid effects on NLR. Additionally, there is a potential for under-ascertainment of PAH cases prior to the 2022 ESC/ERS guidelines; "grey zone" patients (mPAP 21–24 mmHg, PVR 2–3 WU) without suspected echocardiographic findings may have been undiagnosed, which could impact prevalence estimates. Nevertheless, our findings provide valuable insights into CTD-PAH phenotypes regarding inflammation and immunity. While cluster analysis is a reliable method in clinical research, the selection of variables must be predefined, introducing assumptions that may affect outcomes.

In conclusion, our cluster analysis identified three distinct CTD-PAH phenotypes: vasculopathic, vasculitic, and mixed, each with varying inflammatory, immune, and hemodynamic profiles. The vasculitic phenotype, marked by higher treat-to-target rates and better prognoses, enhances our understanding of the diverse CTD-PAH spectrum and the underlying pathophysiological mechanisms involved.

Supplementary Information

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Supplementary Material 1

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Author contributions
QW: Writing—original draft; methodology; Visualization; DL: Writing—original draft; methodology; Visualization; HY: Writing—original draft; methodology; Data curation; ZZ: Data curation; Investigation; Validation; YZ: Data curation; Investigation; Validation; MZ: Supervision; Writing—review and editing; XS: Supervision; Writing—review and editing; QW: Supervision; Writing—review and editing.

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Data availability
Data is provided within the manuscript or supplementary information files.

Declarations

Ethics approval and consent to participate
The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). This study was approved by the Medical Ethics Committee of the First Affiliated Hospital of Nanjing Medical University (number 2018-SR-333). All prospective participants had given written informed consent.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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