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ORIGINAL RESEARCH

Threshold-Triggered Immune Dysregulation Precedes Clinical COPD: A Stage-Specific Diagnostic Model Combining Cytokine Profiling and Lymphocyte Phenotyping

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Purpose: To establish a stage-specific diagnostic model for chronic obstructive pulmonary disease (COPD) high-risk individuals by characterizing immune dysregulation through integrated cytokine and lymphocyte profiling.

Methods: In this cross-sectional study, 116 participants (34 healthy controls, 56 high-risk individuals, and 26 stable COPD patients) underwent comprehensive immunological evaluation. Peripheral blood cytokine levels (IL-6, IL-8, TNF-α) and lymphocyte subsets—including programmed cell death protein 1-positive (PD-1$^+$) CD4$^+$ T cells and effector memory regulatory T cells (emTregs)—were quantified via flow cytometry and multiplex immunoassays. A multivariable logistic regression model was developed to identify predictors of high-risk COPD, incorporating variables selected through hierarchical likelihood ratio testing and variance inflation factor (VIF)-based multicollinearity adjustment. Statistical validation included receiver operating characteristic (ROC) curve analysis, sensitivity-specificity assessment, and effect size calculations (Cohen's f).

Results: Threshold-driven immunological alterations were identified in high-risk individuals, marked by a 7.8-fold elevation in PD-1$^+$CD4$^+$ T cells ($p < 0.001$) and increased IL-6 levels (median difference: 1.064 pg/mL, $p < 0.001$). Effector memory Tregs exhibited progressive depletion from healthy to stable COPD stages ($p < 0.001$). The final regression model—incorporating PD-1$^+$CD4$^+$ T cells, age, and emTregs-demonstrated robust diagnostic accuracy (*AUC* = 0.912; 95% *CI*: 0.848–0.975), with 80.9% sensitivity and 79.3% specificity. PD-1+CD4+ T cells and age independently predicted high-risk status (adjusted odds ratio = 1.17, 95% *CI*: 1.05–1.30, $p = 0.005$; adjusted odds ratio = 1.11, 95% *CI*: 1.03–1.20, $p = 0.007$).

Conclusion: This study delineates a threshold-triggered immune signature preceding clinical COPD, providing a validated diagnostic framework for early detection. By integrating lymphocyte exhaustion markers and cytokine dynamics, the model bridges a critical gap in identifying subclinical immune dysfunction, enabling targeted interventions prior to irreversible lung damage.

Keywords: chronic obstructive pulmonary disease, immune dysregulation, T-cell exhaustion, diagnostic model, biomarker discovery

Introduction

Chronic obstructive pulmonary disease (COPD), the third leading cause of death globally, is characterized by chronic airway inflammation and irreversible airflow limitation. However, most patients are diagnosed only after significant symptoms emerge, such as marked declines in lung function, missing the optimal window for early intervention.[1–3] Epidemiological data indicate that approximately 46% of COPD patients require hospitalization due to acute exacerbations, underscoring the urgent need for early detection in high-risk individuals—those with preserved lung function but persistent inflammation due to smoking or environmental exposures.[4] Current diagnostic reliance on spirometry (eg,

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FEV1/FVC ratio) fails to capture early immune dysregulation, delaying therapeutic interventions. Consequently, identifying dynamic biomarkers reflecting immunological aberrations in the high-risk stage has become a critical challenge in COPD precision management.[5,6]

Emerging evidence highlights the central role of immune dysregulation in COPD pathogenesis. Pro-inflammatory cytokines such as IL-6, which exacerbates airway inflammation via STAT3 activation and disrupts alveolar epithelial integrity, are elevated even in high-risk individuals.[7–9] Aberrant B-cell subsets, including transitional B cells, may drive lymphoid follicle formation and autoantibody production, a phenomenon observed in early COPD.[10–12] Furthermore, functional impairment of regulatory T cells (Tregs)—particularly the decline in effector memory Treg (emTreg) suppressive capacity—and concomitant Th17 hyperactivation create a self-perpetuating cycle of inflammation and immune suppression, accelerating disease progression.[13,14] These findings suggest that dynamic immune biomarkers could serve as a critical tool for identifying high-risk individuals before irreversible lung damage occurs.[15,16]

The role of immune dysregulation in early COPD pathogenesis is further supported by mechanistic insights into specific biomarkers. For instance, PD-1+ T cell exhaustion has been shown to suppress antigen-specific immune responses, leading to increased bacterial colonization in the airways.[17] IL-6 promotes airway smooth muscle proliferation and accelerates airflow limitation through STAT3 signaling.[7] Impaired emTreg function exacerbates Th17 -mediated neutrophilic inflammation, contributing to lung tissue damage.[14] Moreover, recent studies indicate that reduced transitional B cell levels are associated with higher exacerbation risk in COPD,[18] and fluctuations in IL-8 between high-risk and stable phases may be linked to airway mucus secretion.[19] These findings reinforce the rationale for selecting IL-6, PD-1+CD4+ T cells, and emTregs as core biomarkers in this study.

Despite advances, significant knowledge gaps persist regarding the stage-specific dynamics of COPD biomarkers.[20] While markers like blood eosinophils and CRP are used clinically, their longitudinal patterns across disease stages remain poorly defined.[21] For instance, IL-8 levels rise sharply during acute exacerbations, but their fluctuations between high-risk and stable phases remain controversial.[22] Most studies focus on single time points or specific stages (eg, exacerbations), lacking cross-stage longitudinal data to validate biomarker utility.[23,24] Crucially, conventional metrics such as FEV1 cannot distinguish early immune-driven pathology from structural lung damage, necessitating integrated multidimensional immune signatures for precise staging.[17,19]

To address these gaps, we hypothesize that threshold-based changes in specific immune markers (eg, IL-6, PD-$1^{+}CD4^{+}$ T cells, and emTregs) during the high-risk phase can form the basis of a cross-stage diagnostic model. Supporting evidence includes: (1) the abrupt 7 - fold increase in PD-$1^{+}CD4^{+}$ T cells in high-risk individuals, suggesting their role as an "immune switch";[25] (2) machine learning studies demonstrating that combining cytokines (eg, IL-6) and lymphocyte subsets (eg, transitional B cells) significantly improves discrimination between high-risk and healthy populations ($AUC > 0.9$);[26,27] (3) the clinical relevance of immune biomarkers in guiding targeted therapies, such as IL - 4Rα inhibitors like dupilumab.[28,29] By delineating the dynamic immunological profiles of high-risk COPD, this study aims to develop a multiparameter diagnostic model to identify actionable intervention windows, offering a novel strategy for early disease prevention and management.

Materials and Methods

Study Design and Participants

This cross-sectional study was conducted between March 2023 and December 2024 in accordance with the Declaration of Helsinki and approved by the Ethics Committee of Shanghai Fifth People's Hospital, Fudan University (Approval No. 2023-153). The ethical approval details can be accessed via the official website of the Ethics Committee of Shanghai Fifth People's Hospital, Fudan University (available upon request by contacting the committee directly). A total of 116 participants were recruited from the Jiangchuan Road Community in Shanghai, including 34 healthy controls, 56 individuals at high risk for COPD, and 26 stable COPD patients. Sample size estimation was performed using G*Power 3.1 based on an effect size of $d = 0.82$ for PD-1+CD4+ T cells derived from previous studies, with $\alpha = 0.05$ and $\beta = 0.2$, yielding a minimum requirement of 30 participants per group. Although the stable COPD group included 26 participants, slightly below the estimated sample size due to challenges in recruiting eligible stable COPD patients

without recent exacerbations from the community, the healthy control and high-risk groups met the statistical power requirement. Inclusion criteria comprised age 35–75 years, local residency ≥ 1 year, and absence of acute respiratory infections within the preceding month. Exclusion criteria included psychiatric disorders, uncontrolled hypertension (systolic/diastolic blood pressure > 200/100 mmHg), recent major surgeries, pregnancy or lactation, severe cardiovascular diseases, and use of immunosuppressive agents. Participant screening involved initial community health examination, followed by COPD Screening Questionnaire (COPD-SQ) assessment and post-bronchodilator spirometry confirmation. Written informed consent was obtained from all participants.

Clinical Assessments and Grouping

Clinical assessments included the COPD-SQ and post-bronchodilator spirometry using the PF680 spirometer (Yiliankang Medical Technology) following American Thoracic Society/European Respiratory Society (ATS/ERS) guidelines. Participants were stratified into three groups: healthy controls (COPD-SQ <16, $FEV_1/FVC \geq 0.7$), high-risk individuals (COPD-SQ ≥ 16, $FEV_1/FVC \geq 0.7$, plus smoking history ≥ 10 pack-years or chronic exposure to dust/chemicals, and ≥2 lower respiratory tract infections in the past year), and stable COPD patients (COPD-SQ ≥ 16, $FEV_1/FVC < 0.7$ without exacerbations in the past 3 months). COPD diagnosis and staging were based on the Global Initiative for Chronic Obstructive Lung Disease (GOLD 2023) criteria. Diffusing capacity for carbon monoxide (DLCO) and lung volumes were not included in this study due to the lack of equipment in the community health center; these will be incorporated in a subsequent validation cohort in a tertiary hospital setting. Patients with acute exacerbations were excluded to minimize the influence of acute inflammation on baseline immune biomarker levels.

Immunological Profiling

Peripheral blood samples were analyzed using the RaiseCyte2L6C flow cytometer (Ruiscike Biotechnology) coupled with a multiplex bead-based immunoassay (BNCBA002-96T, Saihan Biotech) to quantify cytokines, including IL-4, IL-5, IL-6, IL-8, TNF-α. Lymphocyte subsets were characterized via BD FACSymphony™, focusing on T-cell exhaustion markers (PD-1+CD4+/CD8+), regulatory T-cell subpopulations (naïve and effector memory Tregs), and B-cell differentiation stages (transitional, memory, and marginal zone B cells). A complete list of all immunological parameters analyzed in this study is provided in Supplementary Table 1. The following fluorochrome-conjugated antibodies were used: CD4-FITC (BD Biosciences, Cat. No. 555346), CD8-PE (BD Biosciences, Cat. No. 555369), PD-1-APC (BD Biosciences, Cat. No. 561276), and Foxp3-PE-Cy7 (BD Biosciences, Cat. No. 560409).

Statistical Analysis

All statistical analyses were performed using R statistical software (version 4.2.0; R Foundation for Statistical Computing). Non-normally distributed immunological parameters were analyzed using non-parametric methods. Intergroup comparisons were conducted through Kruskal–Wallis tests followed by Dunn's post hoc analysis with Bonferroni correction, with statistical significance defined as adjusted $p < 0.05$. Clinical relevance of observed differences was quantified using Cohen's f effect size, with values exceeding 0.05 considered clinically meaningful.

For binary outcome analysis, we established a multivariable logistic regression framework through rigorous model development. First, univariable logistic regression was performed for all candidate predictors to assess their individual associations with the outcome. Subsequently, potential multicollinearity among predictors was assessed using variance inflation factors (VIF), with variables demonstrating $VIF > 10$ excluded from subsequent modeling. Model selection employed a hierarchical approach using likelihood ratio tests, retaining variables with a univariable $p < 0.10$ for initial consideration in the multivariable model and applying a criterion of $p < 0.05$ for final retention. The final model's diagnostic performance was validated through comprehensive evaluation of sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV).

To address potential concerns regarding sample size and model stability, internal validation was performed using 1000 bootstrap resamples. The bootstrapped AUC median was 0.908 (95% CI: 0.836–0.969), which was highly consistent with the original model (AUC = 0.912), indicating good robustness. Furthermore, the Events Per Variable (EPV) was calculated based on 56 events (high-risk cases) and 5 variables included in the final model, yielding an EPV of 11.2, which satisfies the common heuristic of EPV ≥ 10 and suggests a low risk of overfitting.

Ethical Considerations

All data were anonymized to ensure participant confidentiality. The study protocol and analytical procedures were rigorously reviewed to align with ethical standards, and all participants received comprehensive information about the study objectives and procedures prior to enrollment.

Results

Stage-Specific Differences in Immunological Profiles

Non-parametric Kruskal–Wallis tests revealed significant differences in 34 immunological parameters among healthy controls, COPD high-risk individuals, and stable COPD patients (Table 1). T-cell dysfunction was highlighted by pronounced variations in PD-1^{+}CD4^{+} T cells ($p < 0.001$, *Cohen's f* = 0.059), naïve Tregs ($p < 0.001$, f=0.072), and effector memory Tregs ($p < 0.001$, f = 0.072), underscoring T-cell exhaustion and regulatory imbalance as central features of COPD progression. Pro-inflammatory cytokines exhibited dynamic changes: IL-6 (median: 2.606 vs 1.542 in controls, $p < 0.001$) and IL-8 (2.204 vs 0.736, $p < 0.001$) levels were significantly elevated in the high-risk group, with IL-8 further increasing in stable patients (5.058, $p < 0.001$), indicating progressive chronic inflammation. B-cell abnormalities emerged early, as transitional B cells (1.45 vs 1.96, $p = 0.002$) and memory B cells (14.1 vs 23.62, $p = 0.03$) were enriched in healthy controls, suggesting impaired B-cell differentiation as a hallmark of early immune dysregulation.

Table 1 Intergroup Comparisons of Immunological Parameters by Kruskal–Wallis Test

Parameter Category	Parameter Name	Healthy (n=34)	High-risk (n=56)	Stable COPD (n=36)	*p*-value	Cohen's f
Demographics	Age (years)	58.5 (52.0, 64.0)	66.0 (63.0, 70.0)	67.0 (62.0, 69.0)	< 0.001	0.083
	Sex (Male, %)	17 (50.00)	25 (45.45)	22 (84.62)	0.003	0.595
	Sex (Female, %)	17 (50.00)	30 (54.55)	4 (15.38)	–	–
	Smoking History (Non-smoker, %)	15 (44.12)	36 (65.45)	9 (34.62)	0.018	0.428
	Smoking History (Smoker, %)	19 (55.88)	19 (34.55)	17 (65.38)	–	–
Cytokines (pg/mL)	Interleukin-5 (IL-5)	1.600 (1.270, 1.788)	3.034 (1.671, 4.125)	2.695 (1.411, 7.093)	< 0.001	0.072
	Interleukin-6 (IL-6)	1.542 (1.157, 1.946)	2.606 (1.959, 3.381)	2.359 (1.705, 4.994)	< 0.001	0.065
	Interleukin-8 (IL-8)	0.736 (0.000, 2.751)	2.204 (1.107, 7.103)	5.058 (0.501, 64.500)	< 0.001	0.069
	Interleukin-4 (IL-4)	1.530 (0.697, 1.664)	0.999 (0.783, 1.146)	1.064 (0.886, 1.164)	0.034	0.039
	Tumor Necrosis Factor-α (TNF-α)	1.193 (0.729, 1.689)	2.052 (1.379, 3.746)	2.020 (1.218, 4.959)	0.005	0.055
Lymphocyte Subsets (%)	Total T Cells	66.045 (59.530, 72.460)	62.500 (50.750, 69.685)	71.655 (62.240, 79.760)	0.004	0.056
	CD4^{+} T Cells	51.130 (42.540, 59.990)	57.060 (47.260, 63.625)	48.050 (43.460, 56.830)	0.043	0.032
	CD4^{+} Central Memory T Cells (CD4+ CM)	42.790 (35.700, 49.600)	48.900 (40.390, 56.845)	43.105 (37.400, 53.760)	0.062	0.034
	CD8^{+} T Cells	36.395 (31.970, 48.220)	36.310 (29.275, 41.970)	42.315 (34.440, 49.550)	0.03	0.043
	CD8^{+} Naive T Cells	18.070 (12.630, 23.500)	16.960 (10.180, 23.925)	9.845 (5.330, 19.350)	0.027	0.037
	CD8^{+} Central Memory T Cells (CD8+ CM)	11.355 (8.480, 18.540)	16.000 (10.640, 22.985)	13.235 (9.610, 18.420)	0.076	0.032
	Double-Negative T Cells (DNT)	5.185 (3.180, 9.210)	4.510 (2.735, 9.005)	3.060 (2.280, 5.180)	0.043	0.031
	Natural Killer Cells (NK Cells)	21.020 (13.930, 28.050)	22.690 (17.940, 32.765)	16.525 (11.120, 25.630)	0.013	0.044
	Natural Killer T Cells (NKT Cells)	3.390 (2.150, 10.960)	4.180 (2.850, 6.640)	10.925 (4.100, 16.000)	0.012	0.047
	Transitional B Cells (TrB)	1.960 (1.180, 3.870)	1.450 (0.930, 2.855)	0.800 (0.300, 1.540)	0.002	0.06
	Memory B Cells (MemB)	23.620 (15.260, 29.520)	14.100 (10.260, 23.270)	17.265 (9.520, 37.020)	0.03	0.038
	Marginal Zone B Cells (MZ B)	15.545 (12.750, 20.070)	11.910 (8.480, 16.330)	8.855 (5.300, 15.280)	0.004	0.041
	CD27- CD4^{+} T Cells	19.375 (14.420, 24.560)	19.980 (12.605, 30.345)	29.590 (15.380, 38.850)	0.085	0.048
	PD-1^{+} CD4^{+} T Cells	1.085 (0.420, 9.660)	8.920 (5.445, 15.710)	9.640 (5.750, 14.450)	< 0.001	0.059
	CD27- CD8^{+} T Cells	51.755 (31.320, 56.560)	44.910 (32.990, 55.385)	62.575 (45.700, 74.040)	0.006	0.056
	CD28- CD8^{+} T Cells	48.940 (36.310, 58.500)	44.060 (34.565, 55.805)	60.560 (45.740, 69.200)	0.009	0.049
	CD28^{+} CD8^{+} T Cells	51.040 (41.460, 63.640)	55.860 (43.890, 65.410)	39.425 (30.830, 54.230)	0.015	0.046

(*Continued*)

Table 1 (Continued).

Parameter Category	Parameter Name	Healthy (n=34)	High-risk (n=56)	Stable COPD (n=36)	*p*-value	Cohen's f
	$CD57^+CD8^+$T Cells	44.155 (35.160, 49.760)	35.230 (23.840, 44.430)	41.185 (24.630, 54.830)	0.061	0.035
	PD-1^+ $CD8^+$ T Cells	4.295 (0.960, 14.200)	10.960 (5.205, 16.155)	7.500 (2.860, 14.360)	0.011	0.021
	T Helper 2 Cells (Th2)	42.995 (36.160, 49.560)	46.570 (39.540, 53.530)	51.430 (40.800, 60.470)	0.015	0.056
	T Helper 17 Cells (Th17)	11.530 (9.810, 15.770)	12.580 (8.930, 16.785)	8.865 (6.870, 11.210)	0.002	0.042
	Naive Regulatory T Cells (nTreg)	22.300 (14.710, 28.080)	14.640 (8.840, 21.775)	14.665 (7.570, 18.880)	< 0.001	0.072
	Effector Memory Regulatory T Cells (emTreg)	77.785 (71.920, 85.290)	85.360 (78.225, 91.130)	85.335 (81.020, 92.430)	< 0.001	0.072

Notes: Data presented as median (IQR). *p*-values adjusted by Bonferroni correction. Effect sizes: $f > 0.07$ indicates clinical significance.

Validation of High-Risk Stage-Specific Biomarkers

Dunn's post hoc analysis of 12 literature-supported biomarkers (Supplementary Table 2) confirmed stage-specific dynamics (Table 2). IL-6 (median difference [Δ] = 1.064, $p < 0.001$) and PD-1^+CD4^+ T cells ($\Delta = 7.835$, $p < 0.001$) were significantly elevated in the high-risk group compared to controls, with no difference between high-risk and stable stages ($p > 0.05$), supporting a threshold-triggered immune dysregulation hypothesis. Age showed strong confounding effects ($\Delta = 7.5$–8.5, $p < 0.001$; *Cohen's d*=1.15 vs $d = 0.82$–0.92 for biomarkers), necessitating integration with biological markers. Progressive declines in transitional B cells ($\Delta = 0.65$, $p = 0.016$) and Th17 cells ($\Delta = 3.865$, $p = 0.003$) from high-risk to stable stages reflected lymphoid hyperplasia and inflammatory dysregulation.

Table 2 Stage-Specific Biomarker Dynamics via Dunn's Post Hoc Test

Marker	Comparison	*p*-value	Δ Median	Cohen's d
IL-6	Healthy vs High-risk	<0.001	1.064	0.921
	Healthy vs Stable	0.001	0.817	0.849
	High-risk vs Stable	1.632	0.247	0.422
IL-8	Healthy vs High-risk	0.001	1.469	0.551
	Healthy vs Stable	0.004	4.322	0.706
	High-risk vs Stable	0.313	2.854	0.754
TNF-α	Healthy vs High-risk	0.002	0.86	0.554
	Healthy vs Stable	0.109	0.827	0.622
	High-risk vs Stable	1.711	0.033	0.497
TrB	Healthy vs High-risk	0.138	0.51	0.515
	Healthy vs Stable	0.003	1.16	0.823
	High-risk vs Stable	0.016	0.65	0.516
PD-1^+ $CD4^+$	Healthy vs High-risk	<0.001	7.835	0.821
	Healthy vs Stable	0.001	8.555	0.849
	High-risk vs Stable	1.895	0.72	0.083
PD-1^+ $CD8^+$	Healthy vs High-risk	0.008	5.995	0.276
	Healthy vs Stable	0.189	3.205	0.048
	High-risk vs Stable	0.434	2.79	0.292
CD28-$CD8^+$	Healthy vs High-risk	1.015	4.88	0.076
	Healthy vs Stable	0.018	11.62	0.586
	High-risk vs Stable	0.010	16.5	0.657
Th17	Healthy vs High-risk	1.987	1.2	0.21
	Healthy vs Stable	0.005	2.665	0.524
	High-risk vs Stable	0.003	3.865	0.487

(*Continued*)

Table 2 (Continued).

Marker	Comparison	*p*-value	Δ Median	Cohen's d
nTreg	Healthy vs High-risk	0.001	7.66	0.865
	Healthy vs Stable	0.001	7.635	1.034
	High-risk vs Stable	1.028	0.025	0.211
emTreg	Healthy vs High-risk	0.001	7.575	0.866
	Healthy vs Stable	0.001	7.55	1.032
	High-risk vs Stable	1.081	0.025	0.206
Age	Healthy vs High-risk	<0.000	7.5	1.15
	Healthy vs Stable	<0.001	8.5	1.076
	High-risk vs Stable	1.903	1	0.003

Notes: Effect size interpretation: $d > 0.8$ indicates strong clinical relevance.

Logistic Regression Model for High-Risk COPD Prediction

A multivariable logistic regression model was developed to predict high-risk COPD status. The model demonstrated excellent discrimination (*AUC* = 0.912, *95% CI*: 0.848–0.975) (Figure 1A), with 80.9% accuracy, 80.9% sensitivity, and 79.3% specificity at the optimal cutoff of 0.5 (Figure 1B).

Variable selection was informed by univariable analysis (Table 3) and assessment of multicollinearity. Naïve Tregs were excluded due to severe collinearity with effector memory Tregs (VIF > 10,000; Supplementary Table 3). The final

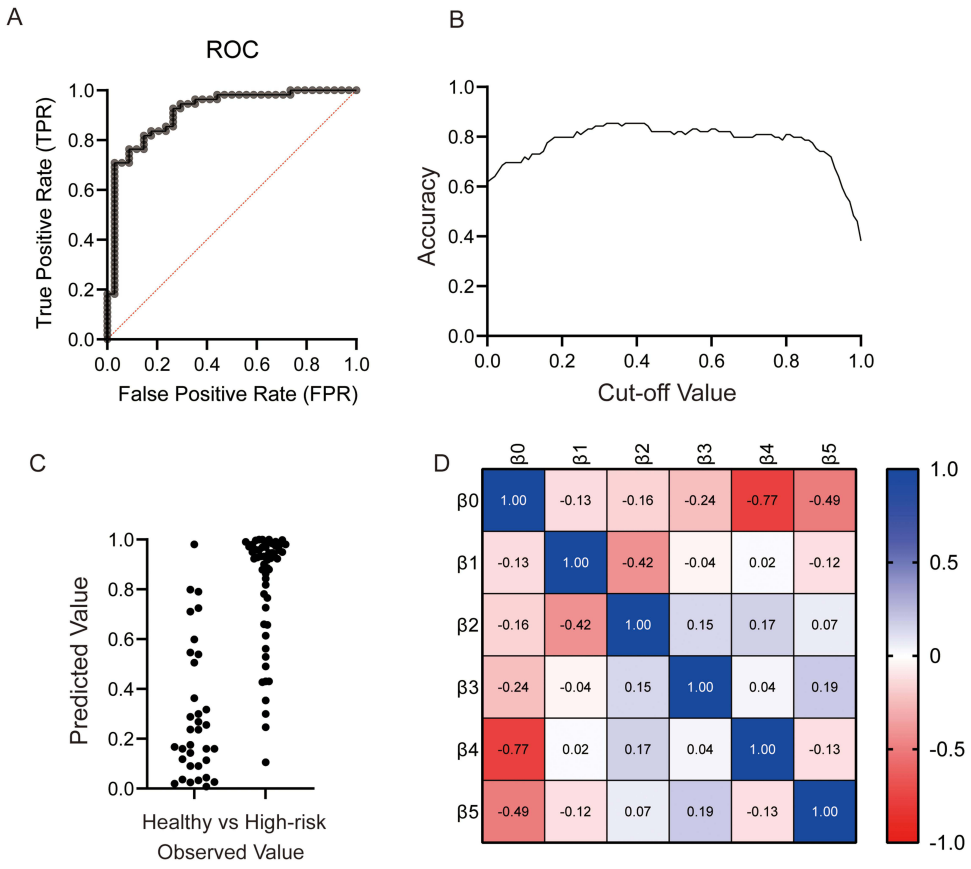

Figure 1 Diagnostic Performance of the COPD Risk Stratification Model. (**A**) ROC curve analysis (*AUC* = 0.912, $p < 0.001$; shaded area=95% CI). (**B**) Optimal cutoff (0.5) balancing sensitivity (80.9%) and specificity (79.3%). (**C**) Observed vs predicted classification agreement (NPV=75.76%, PPV=83.93%). (**D**) Covariance heatmap of model variables ($p < 0.05$ marked with asterisks).

Table 3 Univariable and Multivariable Logistic Regression Analysis for Predicting High-Risk COPD Status

Variable	Unadjusted OR (95% CI)	*p*-value	Adjusted OR (95% CI)	*p*-value
IL-6	1.16 (1.08–1.26)	< 0.001	1.76 (0.77–4.01)	0.18
IL-8	3.26 (1.84–6.51)	< 0.001	1.1 (0.97–1.24)	0.13
PD-1^{+} CD4^{+}	1.11 (1.03–1.26)	0.036	1.17 (1.05–1.30)	0.005
emTreg	1.14 (1.06–1.24)	0.001	1.09 (1.01–1.18)	0.032
Age	1.09 (1.04–1.16)	0.001	1.11 (1.03–1.20)	0.007

Table 4 Model Performance Metrics

Accuracy	Sensitivity	Precision	F1-Score	AUC (95% CI)
0.809	0.809	0.808	0.808	0.912 (0.848–0.975)

model, incorporating IL-6, IL-8, PD-1+CD4+ T cells, effector memory Tregs, and age, exhibited a good fit (Likelihood ratio $\chi^2 = 63.784$, $p < 0.001$) (Supplementary Table 4).

In the multivariable analysis (Table 4), PD-1+CD4+ T cells (*adjusted OR* = 1.17, 95% CI: 1.05–1.30, $p = 0.005$) and age (*adjusted OR* = 1.11, *95% CI*: 1.03–1.20, $p = 0.007$) were identified as independent predictors. Effector memory Tregs showed a marginal association (*adjusted OR* = 1.09, *95% CI*: 1.01–1.18, $p = 0.032$). IL-6 and IL-8 were not independently associated with the outcome ($p > 0.05$).

The model yielded a negative predictive value of 75.76% and a positive predictive value of 83.93% (Figure 1C). Covariance analysis confirmed general independence among predictor variables, with a moderate correlation between IL-6 and IL-8 ($r = 0.42$) (Figure 1D).

Discussion

Exhibit a robust discriminative capacity (*AUC* = 0.912) for the identification of individuals at high risk. Notably, conventional spirometry (FEV1/FVC ≥ 0.7) is unable to detect subclinical immune dysfunction, whereas the proposed model overcomes this limitation by integrating immune markers.

The substantial increase in PD-1+CD4+ T cells (7.8-fold, $p < 0.001$) strengthens their role as an early indicator of immune exhaustion, which is consistent with their hypothesized function as an "immune switch" in the early pa[30,31] thogenesis of chronic obstructive pulmonary disease (COPD). The absence of a significant difference between the high-risk and stable COPD groups ($p > 0.05$) further implies that PD-1+CD4+ T cells mark a threshold of immune dysregulation rather than disease progression.

In comparison with previously reported biomarkers associated with COPD risk and exacerbations, such as eosinophils, CRP, and IL-8 during acute episodes, our panel offers distinct advantages in detecting pre-clinical immune dysregulation.[22–24] For example, while IL-8 is well-established in exacerbations,[22] its fluctuations during the stable and high-risk phases remain controversial. In contrast, PD-1+CD4+ T cells and emTregs exhibit consistent stage - specific alterations, providing more stable predictive value. Moreover, unlike systemic inflammatory markers such as CRP, which lack cellular specificity, our model incorporates lymphocyte subsets that reflect adaptive immune malfunction, in line with the recent emphasis on lymphoid follicle formation and autoimmune mechanisms in early COPD.[10–12]

The stage-specific dynamics of IL-6 and IL-8 further emphasize their utility in tracking inflammatory progression. While the elevation of IL-6 in high-risk individuals (median $\Delta = 1.064$, $p < 0.001$) reflects early signal transducer and activator of transcription 3 (STAT3)-mediated epithelial disruption,[32,33] the marked increase in IL-8 in stable COPD ($\Delta = 4.322$ pg/mL, $p = 0.004$) is consistent with sustained neutrophilic inflammation. The decline in TrB) from the high-risk to

stable stages ($\Delta = 0.65\%$, $p = 0.016$) underscores defective B-cell regulation, supporting recent hypotheses on B-cell-mediated autoimmunity in COPD progression.[34,35]

The final logistic model retained PD-1+CD4+ T cells and age as independent predictors (OR = 1.17 and 1.11, $p < 0.01$), highlighting the convergence of immunosenescence and T-cell exhaustion in early COPD. The marginal contribution of emTregs (OR = 1.09, $p = 0.032$) may reflect their complex role in balancing inflammation and suppression, particularly in the context of Th17 hyperactivity.[10,36] It is notable that the exclusion of IL-6 and IL-8 did not significantly,[10,36] impair model performance, suggesting that cellular exhaustion markers may offer greater reliability than soluble inflammatory mediators for early detection.

Clinical Implications of These Biomarkers Include

PD-1+CD4+ T cells may serve as a screening instrument to identify high-risk individuals requiring closer surveillance; Elevated IL-6 may indicate early epithelial injury and inform targeted anti-inflammatory intervention; The emTreg/Th17 imbalance may assist in stratifying progression risk and open prospects for exploring immunomodulators (eg, IL-17 inhibitors) in pre-COPD stages. However, these biomarkers have not yet been validated for direct therapeutic guidance, and future randomized controlled trials (RCTs) are necessary to evaluate their utility in clinical decision-making.

Conclusions

This study exhibits several merits. Specifically, it involves the development of a novel integrated diagnostic model that combines cytokine profiling and lymphocyte phenotyping for the identification of high-risk COPD individuals. This model achieved an area under the curve (AUC) of 0.912 and demonstrated superior performance compared to single biomarkers. Moreover, the community-based recruitment strategy enhances the representativeness of the sample and the clinical applicability in screening scenarios.

Nonetheless, several limitations should be recognized. The single-center cross-sectional design prevents the assessment of the longitudinal predictive value of these biomarkers. The relatively small sample size in the stable COPD group (n = 26) may impact the stability of inter-group comparisons. Additionally, the absence of diffusing capacity of the lung for carbon monoxide (DLCO) and lung volume measurements restricts the comprehensiveness of the diagnostic evaluation. Finally, the relationship between biomarkers and treatment response was not investigated; thus, further validation is required to determine the clinical translatability.

Although this study offers cross-sectional validation of stage-specific biomarkers, longitudinal cohorts are necessary to confirm their predictive value for COPD incidence and exacerbations. The inclusion of age as a confounder, despite statistical adjustment, emphasizes the need for age-stratified analyses in larger populations. Future research should integrate multi-omics data (eg, transcriptomics of PD-1+CD4+ subsets) to refine the mechanistic links between immune dysregulation and airflow limitation.

In summary, our model promotes COPD precision medicine by identifying high-risk individuals prior to the occurrence of irreversible lung damage. The threshold-based immune signatures proposed herein not only fill a crucial diagnostic gap but also provide a framework for immunomodulatory trials aimed at intercepting COPD at its earliest stages.

Data Sharing Statement

The datasets generated and analyzed during the current study are not publicly available due to ethical restrictions and participant confidentiality agreements. However, anonymized data supporting the findings of this study are available from the corresponding author upon reasonable request, subject to institutional review board approval.

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Disclosure

The authors report no conflicts of interest in this work.

References

1. Melen E, Faner R, Allinson JP, et al. Lung-function trajectories: relevance and implementation in clinical practice. *Lancet*. 2024;403 (10435):1494–1503. doi:10.1016/S0140-6736(24)00016-3
2. Qu S, Feng E, Dong D, et al. Early screening of lung function by electrical impedance tomography in people with normal spirometry reveals unrecognized pathological features. *Nat Commun*. 2025;16(1):622. doi:10.1038/s41467-024-55505-2
3. Sun Y, Zhou J. New insights into early intervention of chronic obstructive pulmonary disease with mild airflow limitation. *Int J Chron Obstruct Pulmon Dis*. 2019;14:1119–1125. doi:10.2147/COPD.S205382
4. Goyal A, Saeed H, Sultan W, et al. Mortality trends and disparities for coexisting chronic obstructive pulmonary disease and cardiovascular disease: a retrospective analysis of deaths in the United States from 1999–2020. *PLoS One*. 2025;20(2):e317592. doi:10.1371/journal.pone.0317592
5. Razia DEM, Gao C, Wang C, et al. Targeting non-eosinophilic immunological pathways in COPD and AECOPD: current insights and therapeutic strategies. *Int J Chron Obstruct Pulmon Dis*. 2025;20:511–532. doi:10.2147/COPD.S506616
6. Chiu S, Chiang T, Chen C. Identification and validation of SPP1 as a potential biomarker for COPD through comprehensive bioinformatics analysis. *Respir Med*. 2025;237:107953. doi:10.1016/j.rmed.2025.107953
7. Kiszalkiewicz JM, Majewski S, Piotrowski WJ, et al. Evaluation of selected IL6/STAT3 pathway molecules and miRNA expression in chronic obstructive pulmonary disease. *Sci Rep*. 2021;11(1):22756. doi:10.1038/s41598-021-01950-8
8. Mariniello DF, D'Agnano V, Cennamo D, et al. Comorbidities in COPD: current and Future Treatment Challenges. *J Clin Med*. 2024;13(3):743. doi:10.3390/jcm13030743
9. Pavord ID, Jones PW, Burgel P, Rabe KF. Exacerbations of COPD. *Int J Chron Obstruct Pulmon Dis*. 2016;11(Spec Iss):21–30. doi:10.2147/COPD.S85978
10. Menon M, Hussell T, Ali Shuwa H. Regulatory B cells in respiratory health and diseases. *Immunol Rev*. 2021;299(1):61–73. doi:10.1111/imr.12941
11. Hu K, Chuang M, Lai C, Liao K. Meta-analysis of randomized, controlled trials assessing the effectiveness and safety of biological treatments in chronic obstructive pulmonary disease patients. *Clin Ther*. 2025;47(3):226–234. doi:10.1016/j.clinthera.2024.12.001
12. Tang W, Rong Y, Zhang H, et al. The correlation between a Th1/Th2 cytokines imbalance and vitamin D level in patients with early chronic obstructive pulmonary disease (COPD), based on screening results. *Front Physiol*. 2023;14:1032786. doi:10.3389/fphys.2023.1032786
13. Alves LHV, Ito JT, Almeida FM, et al. Phenotypes of regulatory T cells in different stages of COPD. *Int Immunopharmacol*. 2024;140:112765. doi:10.1016/j.intimp.2024.112765
14. Thomas R, Qiao S, Yang X. Th17/Treg imbalance: implications in lung inflammatory diseases. *Int J Mol Sci*. 2023;24(5):4865. doi:10.3390/ijms24054865
15. Pantazopoulos I, Magounaki K, Kotsiou O, et al. Incorporating biomarkers in COPD management: the research keeps going. *J Pers Med*. 2022;12 (3):379. doi:10.3390/jpm12030379
16. Zhao X, Yue Y, Wang X, Zhang Q. Bioinformatics analysis of PLA2G7 as an immune-related biomarker in COPD by promoting expansion and suppressive functions of MDSCs. *Int Immunopharmacol*. 2023;120:110399. doi:10.1016/j.intimp.2023.110399
17. Wang L, Cai Y, Garssen J, Henricks PAJ, Folkerts G, Braber S. The bidirectional gut-lung axis in chronic obstructive pulmonary disease. *Am J Respir Crit Care Med*. 2023;207(9):1145–1160. doi:10.1164/rccm.202206-1066TR
18. Qi Y, Yan Y, Tang D, et al. Inflammatory and immune mechanisms in COPD: current status and therapeutic prospects. *J Inflamm Res*. 2024;17:6603–6618. doi:10.2147/JIR.S478568
19. Bhat TA, Panzica L, Kalathil SG, Thanavala Y. Immune dysfunction in patients with chronic obstructive pulmonary disease. *Ann Am Thorac Soc*. 2015;12(Suppl 2):S169–S175. doi:10.1513/AnnalsATS.201503-126AW
20. Xu Y, Zhang L, Zhu L, et al. Prognostic value of biomarkers in chronic obstructive pulmonary disease: a comprehensive review. *Int J Chron Obstruct Pulmon Dis*. 2025;20:3123–3134. doi:10.2147/COPD.S531935
21. Greulich T, Mager S, Lucke T, et al. Longitudinal stability of blood eosinophil count strata in the COPD COSYCONET cohort. *Int J Chron Obstruct Pulmon Dis*. 2018;13:2999–3002. doi:10.2147/COPD.S165909
22. Zhang J, Bai C. The significance of serum interleukin-8 in acute exacerbations of chronic obstructive pulmonary disease. *Tanaffos*. 2018;17 (1):13–21.
23. Dhamodharan P, Pillai GG, Arumugam M. Identification of potential phytochemical inhibitors for the proven six chronic obstructive pulmonary disease biomarkers - a molecular dynamics study. *J Biomol Struct Dyn*. 2024;42(20):10994–11019. doi:10.1080/07391102.2023.2259490
24. Sharma G, Banerjee R, Srivastava S. Molecular mechanisms and the interplay of important chronic obstructive pulmonary disease biomarkers reveals novel therapeutic targets. *ACS Omega*. 2023;8(49):46376–46389. doi:10.1021/acsomega.3c07480
25. Xiong X, Zhu M, Wu H, Wu Z, Fan L, Cheng D. T-cell immune status in patients with acute exacerbation of chronic obstructive pulmonary disease: a case-control study. *Front Med*. 2025;12:1433844. doi:10.3389/fmed.2025.1433844
26. Pinarlik F, Babuccu G, Albayrak O, et al. Predictive role and clinical value of serum cytokines in COVID-19. *Infect Dis Clin Microbiol*. 2024;6 (4):258–267. doi:10.36519/idcm.2024.367
27. Onuk S, Sipahioglu H, Karahan S, et al. Cytokine levels and severity of illness scoring systems to predict mortality in COVID-19 infection. *Healthcare*. 2023;11(3):387. doi:10.3390/healthcare11030387

28. Yeh CF, Lan MY, Lin CC, Hung YW, Huang WH, Lai YL. Dual blockade of IL-4 and IL-13 with dupilumab ameliorates sensorineural olfactory dysfunction in mice with eosinophilic sinonasal inflammation. *Rhinology*. 2025;63(1):32–42. doi:10.4193/Rhin24.114
29. Ragnoli B, Morjaria J, Pignatti P, et al. Dupilumab and tezepelumab in severe refractory asthma: new opportunities. *Ther Adv Chronic Dis*. 2022;13:374145903. doi:10.1177/20406223221097327
30. Xue H, Lan X, Xue T, et al. PD-1(+) T lymphocyte proportions and hospitalized exacerbation of COPD: a prospective cohort study. *Respir Res*. 2024;25(1):218. doi:10.1186/s12931-024-02847-6
31. Cao X, Wang Y, Chen Y, et al. Advances in traditional Chinese medicine for the treatment of chronic obstructive pulmonary disease. *J Ethnopharmacol*. 2023;307:116229. doi:10.1016/j.jep.2023.116229
32. Hirano T. IL-6 in inflammation, autoimmunity and cancer. *Int Immunol*. 2021;33(3):127–148.
33. Garbers C, Rose-John S. Dissecting Interleukin-6 classic and trans-signaling in inflammation and cancer. *Methods Mol Biol*. 2023;2691:207–224.
34. Ran B, Qin J, Wu Y, Wen F. Causal role of immune cells in chronic obstructive pulmonary disease: mendelian randomization study. *Expert Rev Clin Immunol*. 2024;20(4):413–421. doi:10.1080/1744666X.2023.2295987
35. Booth S, Hsieh A, Mostaco-Guidolin L, et al. A single-cell atlas of small airway disease in chronic obstructive pulmonary disease: a cross-sectional study. *Am J Respir Crit Care Med*. 2023;208(4):472–486. doi:10.1164/rccm.202303-0534OC
36. Sekiguchi A, Shimokawa C, Kato T, et al. Inhibition of skin fibrosis via regulation of Th17/Treg imbalance in systemic sclerosis. *Sci Rep*. 2025;15(1):1423. doi:10.1038/s41598-025-85895-2