

Identification of abnormal expression profile of peripheral blood miRNA and its diagnostic value in grading the severity of COPD

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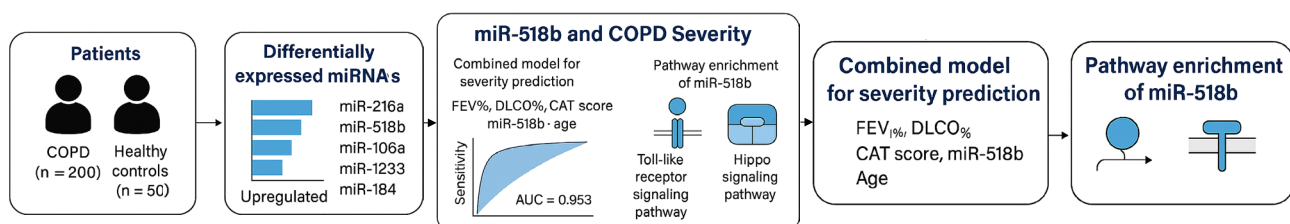
Background. Chronic obstructive pulmonary disease (COPD) is now a major contributor to illness and death on a global scale. Traditional classifications based on pulmonary-function variables like the FEV1 (% predicted), lack sensitivity to early inflammatory changes and disease heterogeneity. In contrast, MiRNAs have emerged as promising biomarkers for respiratory conditions including COPD. The aim of this study was to examine the association between selected MiRNAs and COPD.

Methods. A total of 200 COPD patients (GOLD I-IV, n=50 each) and 50 healthy controls were enrolled. Differentially expressed miRNAs were identified from the GEO database and validated by RT-qPCR. Univariate analysis and LASSO regression were used for feature selection. A logistic regression model incorporating selected variables was established for the forecast COPD severity. KEGG pathway enrichment of miR-518b target genes was performed using DAVID.

Results. Five miRNAs (miR-216a, miR-518b, miR-106a, miR-1233, and miR-184) were substantially increased in COPD and correlated with disease severity ($P<0.001$). LASSO regression identified FEV1 (% predicted), DLCO (% predicted), CAT score, miR-518b, and age as key predictors. The combined model showed excellent classification performance (AUC=0.953; sensitivity=92.9%; specificity=81.3%). MiR-518b emerged as a strong independent risk factor (OR=18.91, $P=0.003$). Gene set enrichment of miR-518b targets pointed to involvement in both the Toll-like receptor and Hippo pathways, implicating its critical roles in inflammation and airway remodeling.

Conclusions. MiR-518b is closely associated with COPD severity and may be useful in clinical practice. A model integrating miRNA expression and clinical parameters provides high predictive value for COPD classification and supports precision diagnosis.

IDENTIFICATION OF ABNORMAL EXPRESSION PROFILE OF PERIPHERAL BLOOD MIRNA AND ITS DIAGNOSTIC VALUE IN GRADING THE SEVERITY OF COPD



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Zhang X. et al., doi: 10.5507/bp.2026.011

Graphical Abstract

Biomedical Papers
<https://biomed.papers.upol.cz>

Key words: COPD, miR-518b, classification performance

Received: October 14, 2025; Revised: April 10, 2026; Accepted: April 14, 2026; Available online: May 4, 2026

<https://doi.org/10.5507/bp.2026.011>

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INTRODUCTION

COPD is a heterogeneous and progressive respiratory disease characterized by persistent airflow limitation and chronic airway inflammation, primarily resulting from long-term exposure to noxious particles or gases, most notably tobacco smoke^{1,2}. According to the GBD data, COPD ranked among the top four causes of death worldwide in 2021, with an increasing clinical and socioeconomic burden, particularly in aging populations³. Despite substantial advances in both pharmacological and non-pharmacological management, early identification and accurate stratification of disease severity remain major clinical challenges.

Spirometry, especially the percentage of predicted forced expiratory volume in one second (FEV1 [% predicted]), remains fundamental for the diagnosis of COPD and for grading the severity of airflow limitation into GOLD stages 1 to 4 (ref.⁴). However, current GOLD recommendations emphasize that clinical classification and treatment stratification are not based on spirometric parameters alone. Instead, patients are primarily categorized into groups A, B, and E according to symptom burden and history of exacerbations, with spirometry serving as a complementary assessment tool¹.

Although spirometric indices provide a standardized approach for grading disease severity, they fail to fully capture the multidimensional nature of COPD, especially in its early or subclinical stages^{5,6}. Moreover, spirometric indices alone do not reflect the underlying molecular and inflammatory processes that drive disease progression, nor do they account for the marked interindividual variability observed in clinical outcomes⁷. This highlights an urgent need for novel biomarkers that can improve risk stratification, monitor disease trajectory, and guide personalized therapeutic strategies.

miRNAs are small non-coding RNAs that modulate gene expression at the post-transcriptional level and are involved in key biological processes relevant to COPD pathogenesis, including immune modulation, oxidative stress, epithelial remodeling, and apoptosis^{8,9}. Owing to their stability in peripheral blood and disease-specific expression patterns, circulating miRNAs have emerged as promising non-invasive biomarkers in various chronic diseases, including pulmonary disorders¹⁰⁻¹². While accumulating evidence implicates dysregulated miRNA expression in COPD development and exacerbations^{13,14}, most studies have focused on single miRNAs or lacked validation across disease stages.

In this study, we systematically identified differentially expressed miRNAs in the peripheral blood of COPD patients using public transcriptomic datasets and validated key candidates by RT-qPCR. We further evaluated their diagnostic and classification performance across GOLD stages and developed an integrated logistic regression model combining miRNA expression profiles with clinical parameters. Pathway enrichment analysis of miRNA target genes was conducted to explore potential molecular mechanisms underlying COPD progression.

MATERIAL AND METHODS

Subjects

The sample size was chosen with reference to existing literature and preliminary experimental data. Assuming a standard deviation of 0.3 for miRNA expression, anticipating a between-group difference of 0.2, with $\alpha = 0.05$ and power $(1-\beta) = 0.80$, the calculated minimum sample size was 36 subjects per group. Considering the recommendation of having 10–15 events per variable in logistic regression modeling, this study ultimately included 50 subjects in the control group and 50 subjects for each GOLD stage of COPD, meeting the sample size requirements for both statistical modeling and intergroup comparisons.

This study was designed as a prospective, observational case-control investigation. During the study period, patients with a clinical diagnosis of COPD were consecutively screened at our hospital according to the 2023 GOLD guidelines. Eligible patients were subsequently stratified based on spirometric severity (GOLD stages I–IV), and 50 patients were enrolled in each stage to ensure balanced representation across disease severity. Enrollment continued until the predefined sample size for each GOLD stage was reached.

Inclusion and exclusion criteria

The inclusion criteria encompassed: (1) individuals aged 40–80 years; (2) Diagnosis of COPD based on the GOLD criteria, specifically when the post-bronchodilator FEV1/FVC ratio was less than 0.70; (3) Currently in a stable phase of COPD without evident symptoms of acute exacerbation; (4) Complete clinical and pulmonary function test data available, allowing for definitive GOLD staging.

The exclusion criteria encompassed: (1) Coexisting respiratory diseases such as lung malignancy, bronchial asthma, interstitial lung disease, or active pulmonary tuberculosis; (2) Presence of systemic autoimmune diseases, like systemic lupus erythematosus, rheumatoid arthritis; (3) History of acute respiratory infection or hospitalization within the past month; (4) History of severe cardiovascular or cerebrovascular disease, hepatic or renal dysfunction, or malignancy; (5) Presence of other diseases or technical issues during sampling or testing that could interfere with blood miRNA detection.

Peripheral blood collection and miRNA extraction

The peripheral venous blood was drawn via the antecubital vein in the early morning under fasting conditions, using EDTA anticoagulant tubes. Following centrifugation at 3,000 rpm, the supernatant was retrieved and kept at -80°C until further use. Total RNA was isolated employing the TRIzol reagent kit. The concentration, along with the purity of RNA was estimated employing a NanoDrop 2000 spectrophotometer, and only samples with an A260/A280 ratio between 1.8 and 2.1 were included in subsequent analyses.

miRNA expression profiling and RT-qPCR validation

In the initial screening phase, peripheral blood miRNA expression data from COPD patients were sourced from the GEO database (GSE24709). The implementation of analyzing differential expression was accomplished via the "limma" package in R, with candidate miRNAs identified.

During the validation phase, the miRNAs identified from the screening stage were further assessed. cDNA synthesis was carried out via TaqMan™ MicroRNA Reverse Transcription Kit, following which was the implementation of RT-qPCR action by means of TaqMan™ MicroRNA Assay Kit on an ABI 7500 Real-Time PCR System. The quantification of miRNA level was accomplished utilizing $2^{-\Delta\Delta C_t}$ method.

Construction of a COPD severity classification model

A binary logistic regression model was developed incorporating miRNA expression levels alongside clinical parameters as predictor indicators, and GOLD stage as the outcome variable (GOLD I-II=0, GOLD III-IV=1). The predictive accuracy of the model was determined through 10-fold cross-validation, with AUC, sensitivity, and specificity reported as performance indicators.

miRNA target gene enrichment analysis

Gene-target prediction for the validated differentially expressed miRNAs was carried out employing the online databases miRDB and TargetScan. Subsequently, KEGG pathway enrichment was implemented via the DAVID platform, with a focus on identifying pathways related to the pathogenesis of COPD, particularly those involved in inflammatory responses and oxidative stress signaling.

Statistical analysis

Data got processed using SPSS version 26.0 as well as R version 4.0.2. Continuous variables were displayed in the manner of mean $\pm$ SD, and ANOVA or indepen-

dent samples t-test was applied for group comparison. Categorical variables were demonstrated as percentages (%), and differences between groups were assessed applying χ^2 test. Variable selection was accomplished utilizing univariate analysis as well as LASSO regression. To evaluate diagnostic efficacy of the model, ROC curve analysis was implemented and key metrics like AUC, sensitivity, specificity and the Youden index were computed. Logistic regression analysis was further applied to assess the prediction worth of each variable for COPD severity. *P*-value less than 0.05 was deemed as statistically significant.

RESULTS

Baseline characteristics

In total, 250 individuals participated in the investigation, comprising 50 healthy controls and 200 patients suffering from COPD. The mild COPD cohort consisted of patients in GOLD stages I-II, while the severe COPD group included those classified as GOLD stages III-IV. Detailed baseline characteristics of all participants were presented in Table 1.

Significant statistically differences among the groups regarding age, smoking index, FEV1 (% predicted), DLCO (% predicted) and CAT scores (all $P < 0.001$) could be inspected. Specifically, as COPD severity increased, both FEV1 (% predicted) and DLCO (% predicted) progressively decreased, while CAT scores showed a corresponding upward trend. These findings suggested that impaired lung function and clinical symptom severity are strongly linked to COPD advancement.

Screening and validation of differentially expressed miRNAs

Analysis of data from the GEO database identified 10 miRNAs exhibiting differential expression in the peripheral blood of COPD patients compared with healthy

Table 1. Baseline characteristics.

Indicators	Control (n=50)	GOLD I (n=50)	GOLD II (n=50)	GOLD III (n=50)	GOLD IV (n=50)	ANOVA F/ χ^2	ANOVA <i>P</i>	t/ χ^2 (Mild v Severe)	<i>P</i> (Mild v Severe)
Age (years)	66.01 $\pm$ 8.03	66.87 $\pm$ 7.02	67.56 $\pm$ 6.51	70.80 $\pm$ 7.11	71.70 $\pm$ 8.36	11.382	<0.001	3.919	<0.001
Male [n (%)]	30 (60.0%)	31 (62.0%)	33 (66.0%)	32 (64.0%)	34 (68.0%)	0.868	0.929	0.088	0.767
BMI kg/m ²	23.1 $\pm$ 2.5	23.4 $\pm$ 2.8	22.8 $\pm$ 2.7	23.1 $\pm$ 3.0	22.7 $\pm$ 3.3	0.126	0.882	0.478	0.633
Smoking index (pack-years)	21.23 $\pm$ 9.30	28.62 $\pm$ 9.87	32.04 $\pm$ 10.68	35.22 $\pm$ 10.91	36.68 $\pm$ 11.47	27.461	<0.001	3.699	<0.001
FEV1 (% predicted)	91.13 $\pm$ 8.94	80.01 $\pm$ 9.77	69.44 $\pm$ 11.52	48.77 $\pm$ 12.01	36.99 $\pm$ 12.55	201.74	<0.001	19.594	<0.001
DLCO (% predicted)	92.85 $\pm$ 5.59	83.01 $\pm$ 10.77	75.75 $\pm$ 13.52	54.36 $\pm$ 13.99	46.55 $\pm$ 13.21	184.31	<0.001	15.858	<0.001
CAT score	6.60 $\pm$ 2.60	8.33 $\pm$ 5.84	11.15 $\pm$ 6.55	20.13 $\pm$ 7.25	22.11 $\pm$ 7.47	126.15	<0.001	11.280	<0.001
Hypertension [n (%)]	8 (16.0%)	10 (20.0%)	13 (26.0%)	14 (28.0%)	13 (26.0%)	2.592	0.274	0.427	0.513
Diabetes [n (%)]	6 (12.0%)	5 (10.0%)	7 (14.0%)	8 (16.0%)	6 (12.0%)	0.364	0.834	0.177	0.674

GOLD stages were defined based on individual post-bronchodilator FEV1 (% predicted) values according to GOLD criteria; values shown represent group-level descriptive statistics.

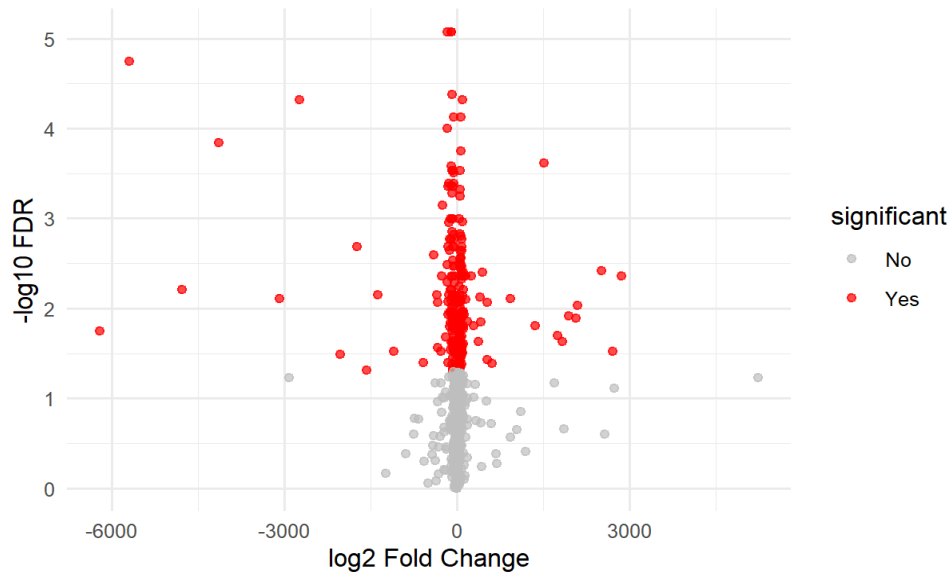


Fig. 1. Volcano plot of differentially expressed miRNAs.

The x-axis represents the $\log_2$ fold change, and the y-axis represents $-\log_{10}$ FDR. Each dot corresponds to a miRNA, with red indicating significantly different expression ($|\log_2FC| > 1$, $FDR < 0.05$) and gray indicating no significant difference.

Table 2. Expression results of differentially expressed miRNAs.

miRNA	Control	GOLD I	GOLD II	GOLD III	GOLD IV	ANOVA_F	ANOVA_P	t (Mild vs Severe)	P (Mild v Severe)
miR-216a	1.03±0.14	1.07±0.19	1.14±0.23	1.27±0.25	1.38±0.27	15.23	<0.001	6.583	<0.001
miR-518b	0.98±0.18	1.11±0.20	1.19±0.22	1.24±0.26	1.31±0.28	14.88	<0.001	3.801	<0.001
miR-106a	1.04±0.19	1.15±0.23	1.21±0.24	1.29±0.27	1.34±0.25	13.95	<0.001	3.957	<0.001
miR-1233	1.03±0.14	1.12±0.17	1.17±0.18	1.25±0.21	1.33±0.22	12.75	<0.001	4.925	<0.001
miR-184	1.01±0.15	1.13±0.21	1.19±0.23	1.27±0.23	1.36±0.26	12.23	<0.001	4.805	<0.001
miR-200a	1.02±0.16	1.04±0.15	1.06±0.18	1.07±0.19	1.08±0.21	1.09	0.364	0.992	0.322
miR-20b	1.05±0.15	1.08±0.17	1.10±0.19	1.11±0.18	1.13±0.21	1.33	0.265	1.115	0.266
miR-224	1.01±0.17	1.05±0.16	1.07±0.18	1.09±0.20	1.11±0.22	1.41	0.236	1.480	0.140
miR-182	1.03±0.16	1.06±0.17	1.10±0.20	1.12±0.21	1.14±0.23	1.56	0.195	1.720	0.087
miR-216b	1.00±0.14	1.02±0.15	1.05±0.17	1.07±0.19	1.08±0.21	1.12	0.342	1.561	0.120

controls (miR-216a, miR-518b, miR-200a, miR-106a, miR-1233, miR-20b, miR-224, miR-182, miR-184, and miR-216b; $|\log_2FC| > 1$, $FDR < 0.05$) (Fig. 1). In the RT-qPCR validation phase, the expression levels of these 10 candidate miRNAs got measured across the control group as well as COPD patients at different GOLD stages (Table 2). An obvious elevation of five miRNAs, including miR-216a, miR-518b, miR-106a, miR-1233, and miR-184 could be traced in COPD sufferers relative to the healthy individuals, with expression levels increasing progressively with disease severity. These differences were statistically significant ($P < 0.001$), implying a credible involvement of these miRNAs in COPD advancement. Conversely, the remaining five miRNAs, including miR-200a, miR-20b, miR-224, miR-182, and miR-216b, did not show appreciable expression changes either among the groups or between the mild and severe COPD cohorts (all $P > 0.05$), indicating limited diagnostic value for disease staging in this study.

Univariate analysis and LASSO regression for variable selection in COPD patients

To further identify key indicators distinguishing between mild (GOLD I-II) and severe (GOLD III-IV) COPD, univariate analysis was first performed on 200 COPD patients. The results showed that age, smoking index, FEV1%, DLCO%, CAT score, and miR-216a, miR-518b, miR-106a, miR-1233, and miR-184 levels were distinctly different in patients suffering from mild or severe COPD (all $P < 0.05$), suggesting these indicators as potential candidate variables for disease severity classification. Subsequently, LASSO regression was applied to further screen variables, aiming to reduce multicollinearity and prevent model overfitting (Fig. 2). Then, 10-fold cross-validation was applied for the determination of the optimal regularization parameter (λ), five variables, including FEV1 (% predicted), DLCO (% predicted), CAT score, miR-518b, and age, were ultimately selected as the optimal feature set for subsequent joint modeling analysis.

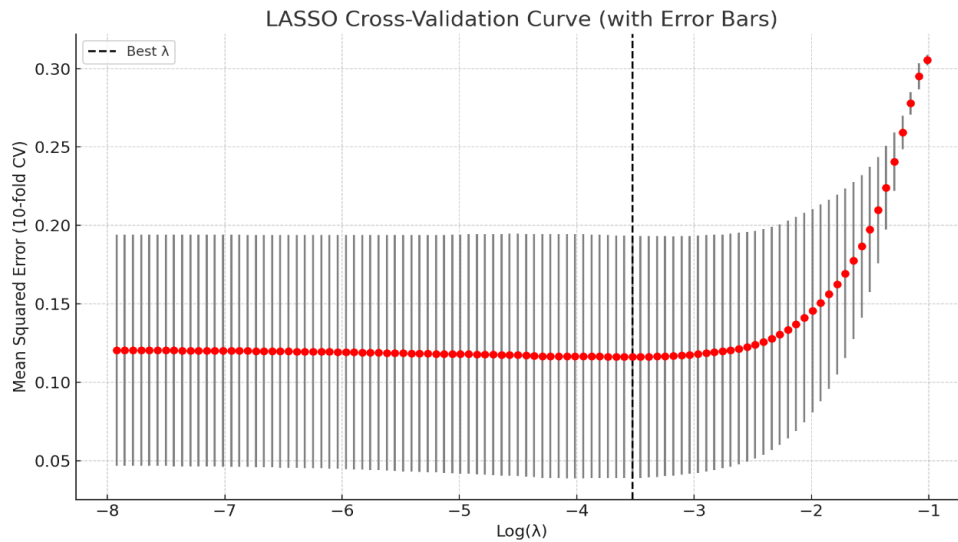


Fig. 2. Feature selection process using LASSO regression.

The x-axis represents the log-transformed λ values, and the y-axis indicates the mean squared error (MSE). Red dots represent the MSE of the model under different λ values, with gray error bars showing the standard error. The black dashed line denotes the optimal λ value corresponding to the minimum MSE.

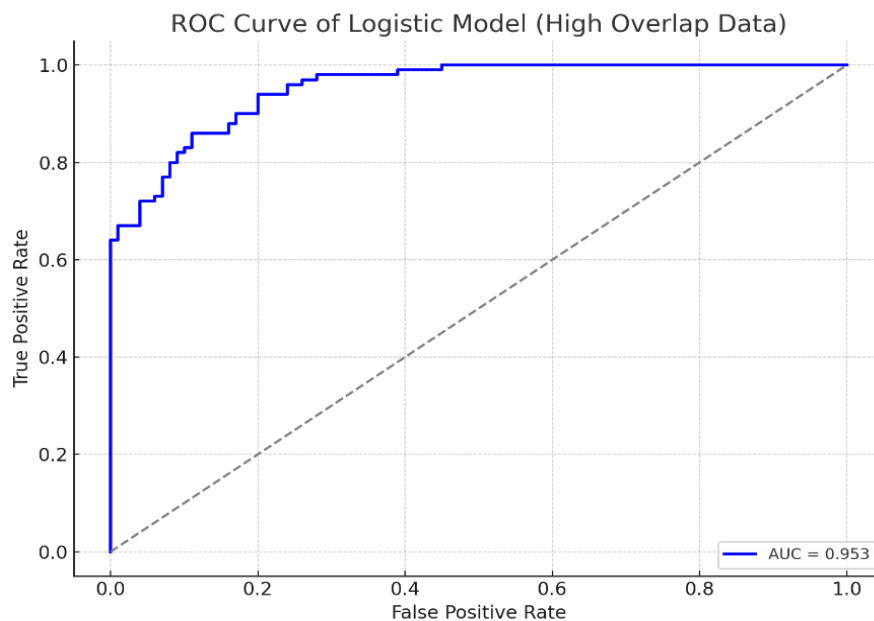


Fig. 3. The performance of the logistic regression model was evaluated using the ROC curve.

Logistic regression-based joint modeling and model performance evaluation

A binary logistic regression model was constructed using the five variables selected by LASSO regression, including FEV1 (% predicted), DLCO (% predicted), CAT score, miR-518b, and age, as independent variables, and GOLD classification (mild: I-II=0; severe: III-IV=1) as the dependent variable. Model performance was assessed using 10-fold cross-validation. The analysis yielded an AUC of 0.953, reflecting excellent discrimination (Fig. 3). The optimal cut-off point for classification was established based on the ROC curve (cutoff=0.513). At this cut-off, the model achieved 92.9% sensitivity, 81.3% specificity, an

accuracy of 86.7% and a Youden index of 0.743, suggesting that the model possesses strong practical value and robustness for COPD severity classification.

The multivariate logistic regression findings were presented in Table 3. FEV1 (% predicted), (OR=0.91, 95% CI: 0.85–0.96, $P<0.001$) and DLCO (% predicted) (OR=0.87, 95% CI: 0.81–0.93, $P<0.001$) were identified as significant protective factors, indicating that lower levels of these pulmonary function parameters go hand-in-hand with an elevated likelihood of severe COPD. The CAT score (OR=1.15, 95% CI: 1.05–1.26, $P=0.004$) was positively associated with disease severity, suggesting that more severe clinical symptoms are linked to a higher GOLD

Table 3. Results of multivariate logistic regression analysis.

Variables	β	OR	95% CI	<i>P</i>
miR-518b	2.94	18.91	2.89–123.68	0.003
FEV1 (% predicted)	−0.096	0.91	0.85–0.96	<0.001
DLCO (% predicted)	−0.142	0.87	0.81–0.93	<0.001
CAT score	0.140	1.15	1.05–1.26	0.004
Age	0.086	1.09	0.99–1.19	0.065

classification. The expression level of miR-518b emerged as a strong risk factor in the model (OR=18.91, 95% CI: 2.89–123.68, $P=0.003$), indicating that its upregulation may be closely associated with severe COPD. Although age showed a positive trend (OR=1.09, $P=0.065$), it did not reach statistical significance in this model.

Pathway enrichment analysis of miR-518b target genes

With the aim of further figuring out the potential molecular mechanisms of miR-518b in the progression of COPD, its target genes were predicted using online databases, we implemented KEGG pathway enrichment analysis via the DAVID platform. The results revealed that miR-518b target genes had abundant existence in the Toll-like receptor pathway as well as the Hippo pathway. The former takes a vital part in the inflammatory reaction in COPD and may have a close connection to pulmonary immune imbalance and airway remodeling. The Hippo signaling pathway is involved in cell proliferation, apoptosis, and tissue regeneration, suggesting that miR-518b may contribute to COPD progression and severity classification through these pathways.

DISCUSSION

MiRNA markers have the advantage of non-invasive acquisition, and its combination with other techniques can significantly improve diagnostic accuracy¹⁵. The existing tools have insufficient sensitivity to early or mild COPD, and more sensitive biomarkers need to be developed. This study systematically explored the diagnostic and classification value of miRNAs in COPD, identifying five miRNAs, particularly miR-518b, as significantly associated with disease severity. Through the integration of molecular and clinical features, we established a logistic regression model with excellent discriminatory performance (AUC=0.953), supporting the utility of miRNAs as minimally invasive biomarkers enabling refined stratification of COPD.

miRNAs take pivotal parts in immunological and inflammatory responses associated with lung diseases, like lung malignancy, COPD, asthma, as well as pulmonary fibrosis^{16,17}. Although only a few studies have specifically explored miRNA regulation within peripheral blood immune cells¹⁸, circulating miRNA signatures in whole blood have already shown promise in identifying COPD patients¹⁹. Previous studies have highlighted several candidates, such as miR-21, miR-146a, and miR-223, which exhibit differential expression in COPD and involved in

inflammatory regulation^{20–22}. Consistent with these findings, our study demonstrated that miR-518b expression increased progressively from GOLD stage I to IV and showed a strong independent association with severe COPD (OR=18.91, $P=0.003$), further supporting the notion that miR-518b could function as a robust biomarker for accurate stratification and severity assessment.

While miR-518b has not been widely reported in the context of COPD, its regulatory role has been implicated in immune response and tissue remodeling in other diseases^{23,24}. In our study, KEGG pathway enrichment of predicted miR-518b target genes revealed significant involvement in the Toll-like receptor pathway and Hippo signaling pathway, both of which have known relevance in COPD pathophysiology^{25,26}. TLR signaling plays a key role in innate immune activation and chronic inflammation in COPD patients, contributing to neutrophilic infiltration and airway remodeling^{25,27}. Meanwhile, the Hippo pathway regulates epithelial cell proliferative, apoptotic, and regenerative capabilities, and its dysregulation bear great responsibility for emphysematous changes in COPD lungs^{26,28}. Evidently, beyond its biomarker potential, miR-518b might function directly in the molecular mechanisms driving COPD advancement.

The predictive model we developed, comprising miR-518b, FEV1%, DLCO%, CAT score, and age, demonstrated strong sensitivity (92.9%) and specificity (81.3%), outperforming models based solely on clinical parameters. These findings support the utility of integrating molecular biomarkers with traditional clinical indices to enhance the classification and prognostic accuracy of COPD. Although previous studies have also shown that miRNAs such as miR-34a, miR-199a-5p, and miR-181a are involved in COPD-related inflammation and structural changes^{29–31}; however, few have focused on miRNA-based models for stratification across GOLD stages, which represents a major novelty and contribution of our study.

Nonetheless, our study carries certain limitations. First, the miRNA validation was performed on a single-center cohort, and external validation using independent populations is warranted to confirm the model's generalizability. Second, although bioinformatic analyses suggested involvement of inflammation-related pathways, the downstream functional targets of miR-518b were not experimentally validated. Further mechanistic studies, including luciferase reporter assays and pathway inhibition experiments, are warranted to clarify how miR-518b influences COPD advancement. Third, the present analysis focused primarily on spirometric severity (GOLD stages 1–4) and did not specifically evaluate miRNA expression in relation

to acute exacerbation history or contemporary GOLD A/B/E clinical groups, which integrate symptom burden and exacerbation risk. Future studies incorporating these clinically relevant dimensions are needed to better align miRNA biomarkers with current GOLD classification and treatment stratification.

CONCLUSION

In summary, this investigation identifies miR-518b as a novel peripheral blood biomarker associated with COPD severity and highlights its potential mechanistic involvement in inflammatory and regenerative signaling. The integrated miRNA-clinical model presents a promising tool for enhancing COPD classification and guiding personalized disease management.

Author contributions: XZ: conceptualization, data curation, formal analysis, writing-original draft; XG: methodology, validation, writing-review and editing; LZ: supervision, project administration, writing-review and editing.

Conflict of interest statement: None declared.

REFERENCES

- Agustí A, Celli BR, Criner GJ, Halpin D, Anzueto A, Barnes P, Bourbeau J, Han MK, Martínez FJ, de Oca MM, Mortimer K, Papi A, Pavord I, Roche N, Salvi S, Sin DD, Singh D, Stockley R, Varela MVL, Wedzicha JA, Vogelmeier CF. Global Initiative for Chronic Obstructive Lung Disease 2023 Report: GOLD Executive Summary. *Respirology* 2023;28(4):316-38. doi: 10.1111/resp.14486
- Ding ZQ, Xiao XR, Li N, Sun C, Shi YJ. Association between *Streptococcus* and chronic obstructive pulmonary disease: A two-sample Mendelian randomization study. *Medicine* 2025;104(28):e43306. doi: 10.1097/MD.00000000000043306
- Zhu Y, Shen T, Guo RX, Liu K, Cao X, Yang XY, Zhang CT. Global, regional, and national burden of young COPD, 1990-2021, with forecasts to 2050: a systematic analysis for the global burden of disease study 2021. *BMC public health* 2025;25(1):276. doi: 10.1186/s12889-025-21414-2
- Ulanova MA. Lysozyme activity in the secretions and blood of children with uncomplicated and complicated forms of acute respiratory viral infections. *Pediatrics* 1983;(2):47-9.
- Li T, Zhou HP, Zhou ZJ, Guo LQ, Zhou LF. Computed tomography-identified phenotypes of small airway obstructions in chronic obstructive pulmonary disease. *Chin Med J (Engl)* 2021;134(17):2025-36. doi: 10.1097/CM9.0000000000001724
- Yin C, Udrescu M, Gupta G, Cheng M, Lihu A, Udrescu L, Bogdan P, Mannino DM, Mihaicuta S. Fractional Dynamics Foster Deep Learning of COPD Stage Prediction. *Adv Sci (Weinh)* 2023;10(12):e2203485. doi: 10.1002/advs.202203485
- David B, Bafadhel M, Koenderman L, De Soyza A. Eosinophilic inflammation in COPD: from an inflammatory marker to a treatable trait. *Thorax* 2021;76(2):188-95. doi: 10.1136/thoraxjnl-2020-215167
- Zeng HZ, Liu XX, Liu PP, Jia SY, Wei GY, Chen G, Zhao L. Exercise's protective role in chronic obstructive pulmonary disease via modulation of M1 macrophage phenotype through the miR-124-3p/ERN1 axis. *Sci Prog* 2025;108(3):368504251360892. doi: 10.1177/00368504251360892
- Khanna V, Singh K. MicroRNAs as promising drug delivery target to ameliorate chronic obstructive pulmonary disease using nano-carriers: a comprehensive review. *Mol Cell Biochem* 2025;480(3):1431-48. doi: 10.1007/s11010-024-05110-0
- Lee J, Na HK, Kim WK. Flexible and Reusable Biochip Platform for miRNA Quantification via Dual-Signal Amplification on 3D Au Substrate. *Anal Chem* 2025;97(28):15208-15. doi: 10.1021/acs.analchem.5c01742
- Yan H, Wen YJ, Tian ZM, Hart N, Han S, Hughes SJ, Zeng Y. A one-pot isothermal Cas12-based assay for the sensitive detection of microRNAs. *Nat Biomed Eng* 2023;7(12):1583-601. doi: 10.1038/s41551-023-01033-1
- Lv J, Xiong X. Extracellular Vesicle microRNA: A Promising Biomarker and Therapeutic Target for Respiratory Diseases. *Int J Mol Sci* 2024;25(17):9147. doi: 10.3390/ijms25179147
- Kim RY, Sunkara KP, Bracke KR, Jarnicki AG, Donovan C, Hsu AC, Ieni A, Beckett EL, Galvão I, Wijnant S, Ricciardolo FL, Di Stefano A, Haw TJ, Liu G, Ferguson AL, Palendira U, Wark PA, Conicx G, Mestdagh P, Brusselle GG, Hansbro PM. A microRNA-21-mediated SATB1/S100A9/NF- κ B axis promotes chronic obstructive pulmonary disease pathogenesis. *Sci Transl Med* 2021;13(621):eaav7223. doi: 10.1126/scitranslmed.aav7223
- Nouws J, Wan F, Finnemore E, Roque W, Kim SJ, Bazan I, Li CX, Skold CM, Dai Q, Yan X, Chioccioli M, Neumeister V, Britto CJ, Sweasy J, Bindra R, Wheelock AM, Gomez JL, Kaminski N, Lee PJ, Sauler M. MicroRNA miR-24-3p reduces DNA damage responses, apoptosis, and susceptibility to chronic obstructive pulmonary disease. *JCI insight* 2021;6(2):e134218. doi: 10.1172/jci.insight.134218
- Moisoiu T, Dragomir MP, Iancu SD, Schallenberg S, Birolo G, Ferrero G, Burghilea D, Stefanu A, Cozan RG, Licarete E, Allione A, Matullo G, Iacob G, Bălint Z, Badea RI, Naccarati A, Horst D, Pardini B, Leopold N, Elec F. Combined miRNA and SERS urine liquid biopsy for the point-of-care diagnosis and molecular stratification of bladder cancer. *Mol Med* 2022;28(1):39. doi: 10.1186/s10020-022-00462-z
- De Smet EG, Mestdagh P, Vandesompele J, Brusselle GG, Bracke KR. Non-coding RNAs in the pathogenesis of COPD. *Thorax* 2015;70(8):782-91. doi: 10.1136/thoraxjnl-2014-206560
- Osei ET, Florez-Sampedro L, Timens W, Postma DS, Heijink IH, Brandsma CA. Unravelling the complexity of COPD by microRNAs: it's a small world after all. *Eur Respir J* 2015;46(3):807-18. doi: 10.1183/13993003.02139-2014
- Xie N, Liu G. ncRNA-regulated immune response and its role in inflammatory lung diseases. *Am J Physiol Lung Cell Mol Physiol* 2015;309(10):L1076-87. doi: 10.1152/ajplung.00286.2015
- Leidinger P, Keller A, Borries A, Huwer H, Rohling M, Huebers J, Lenhof HP, Meese E. Specific peripheral miRNA profiles for distinguishing lung cancer from COPD. *Lung cancer* 2011;74(1):41-7. doi: 10.1016/j.lungcan.2011.02.003
- Sai XY, Qin C, Zhang ZX, Yu HD, Bian T. A miRNA-21-Mediated PTEN/Akt/NF- κ B Axis Promotes Chronic Obstructive Pulmonary Disease Pathogenesis. *Int J Chron Obstruct Pulmon Dis* 2024;19:1141-51. doi: 10.2147/COPD.S453593
- Nadi E, Geramirad G, Kahramfar Z, Rasuli-Saravani A, Solgi G. Peripheral Blood Expressions of MicroRNA-146a and MicroRNA-218 in Chronic Obstructive Pulmonary Disease with/without Cigarette Smoke Exposure. *Iran J Allergy Asthma Immunol* 2022;21(4):399-406. doi: 10.18502/ijaa.v21i4.10287
- Roffel MP, Maes T, Brandsma CA, van den Berge M, Vanaudenaerde BM, Joos GF, Brusselle GG, Heijink IH, Bracke KR. MiR-223 is increased in lungs of patients with COPD and modulates cigarette smoke-induced pulmonary inflammation. *Am J Physiol Lung Cell Mol Physiol* 2021;321(6):L1091-L104. doi: 10.1152/ajplung.00252.2021
- Liu M, Wang YQ, Lu HF, Wang H, Shi XM, Shao X, Li YX, Zhao YY, Wang YL. miR-518b Enhances Human Trophoblast Cell Proliferation Through Targeting Rap1b and Activating Ras-MAPK Signal. *Front Endocrinol (Lausanne)* 2018;9:100. doi: 10.3389/fendo.2018.00100
- Yang WM, Lu ZY, Zhi ZF, Liu LL, Deng LJ, Jiang XL, Pang LH. Increased miRNA-518b inhibits trophoblast migration and angiogenesis by targeting EGR1 in early embryonic arrest. *Biol Reprod* 2019;101(4):664-74. doi: 10.1093/biolre/iox109
- Zhang Y, Hou HY, Sui WW, Liu YM, Zeng QL, Li YY, Li C, Zhou H, Zhang YM. Impact of ATP Synthase Subunit β on TLR Signaling Pathway in Promoting Airway Remodeling and Heterogeneity of Small Airway Epithelial Cells in Chronic Obstructive Pulmonary Disease. *J Innate Immun* 2025;17(1):1-27. doi: 10.1159/000547329
- Yeh LY, Fang YT, Lee HS, Liu CH, Chen YY, Lo YC, Laiman V, Liou JP, Chung KF, Chuang HC, Lin CH. A Potent Histone Deacetylase Inhibitor MPT0E028 Mitigates Emphysema Severity via Components of the Hippo Signaling Pathway in an Emphysematous Mouse Model. *Front Med (Lausanne)* 2022;9:794025. doi: 10.3389/fmed.2022.794025

27. Sabroe I, Whyte MK. Toll-like receptor (TLR)-based networks regulate neutrophilic inflammation in respiratory disease. *Biochem Soc Trans* 2007;35(Pt 6):1492-5. doi: 10.1042/BST0351492
28. Tang WF, Li M, Yangzhong XT, Zhang XF, Zu AJ, Hou YJ, Li L, Sun, SB. Hippo signaling pathway and respiratory diseases. *Cell Death Discov* 2022;8(1):213. doi: 10.1038/s41420-022-01020-6
29. Xie LH, Wu MH, Lin H, Liu C, Yang HH, Zhan J, Sun SH. An increased ratio of serum miR-21 to miR-181a levels is associated with the early pathogenic process of chronic obstructive pulmonary disease in asymptomatic heavy smokers. *Mol Biosyst* 2014;10(5):1072-81. doi: 10.1039/c3mb70564a
30. Velasco-Torres Y, Ruiz-López V, Pérez-Bautista O, Buendía-Roldan I, Ramírez-Venegas A, Pérez-Ramos J, Falfán-Valencia R, Ramos C, Montaña M. miR-34a in serum is involved in mild-to-moderate COPD in women exposed to biomass smoke. *BMC Pulm Med* 2019;19(1):227. doi: 10.1186/s12890-019-0977-5
31. Chatila WM, Criner GJ, Hancock WW, Akimova T, Moldover B, Chang JK, Cornwell W, Santerre M, Rogers TJ. Blunted expression of miR-199a-5p in regulatory T cells of patients with chronic obstructive pulmonary disease compared to unaffected smokers. *Clin Exp Immunol* 2014;177(1):341-52. doi: 10.1111/cei.12325