GAWIN, Konrad, ZAWIŚLAK, Wiktoria, IGNASIAK, Anita, CISOWSKI, Michal, DĄBROWSKA, Maria, RYCHLICA, Kacper, CHOLEWIŃSKA-RYCHLICA, Jolanta, MADURA, Paulina and MROZIK-GAŁECKA, Daria. New Biomarkers of Chronic Obstructive Airway Diseases: Impact on Differential Diagnosis, Therapeutic Strategies, and Prognosis in COPD. A literature review. Quality in Sport. 2026;49:67995. eISSN 2450-3118.
https://doi.org/10.12775/QS.2026.49.67995
https://apcz.umk.pl/QS/article/view/67995

The journal has been awarded 20 points in the parametric evaluation by the Ministry of Higher Education and Science of Poland. This is according to the Annex to the announcement of the Minister of Higher Education and Science dated 05.01.2024, No. 32553. The journal has a Unique Identifier: 201398. Scientific disciplines assigned: Economics and Finance (Field of Social Sciences); Management and Quality Sciences (Field of Social Sciences).
Punkty Ministerialne z 2019 - aktualny rok 20 punktów. Załącznik do komunikatu Ministra Szkolnictwa Wyższego i Nauki z dnia 05.01.2024 Lp. 32553. Posiada Unikatowy Identyfikator Czasopisma: 201398. Przypisane dyscypliny naukowe: Ekonomia i finanse (Dziedzina nauk społecznych); Nauki o zarządzaniu i jakości (Dziedzina nauk społecznych).
The authors declare that there is no conflict of interest regarding the publication of this paper.
Received: 05.01.2026. Revised: 15.01.2026. Accepted: 15.01.2026. Published: 18.01.2026.

New Biomarkers of Chronic Obstructive Airway Diseases: Impact on Differential Diagnosis, Therapeutic Strategies, and Prognosis in COPD. A literature review

Konrad Gawin
Central Clinical Hospital of the Medical University of Łódź
Ul. Pomorska 251, 92-213 Łódź, Poland
https://orcid.org/0009-0007-2242-4356
konrad.gawin1@icloud.com

Wiktoria Zawiślak
Dr Karol Jonscher Municipal Medical Center,
Ul. Milionowa 14, 93-113 Łódź
https://orcid.org/0009-0009-2028-8885
zawislak.wiktoria@gmail.com

Anita Ignasiak
Central Clinical Hospital of the Medical University of Łódź
ul. Pomorska 251, 92-213 Łódź, Poland
https://orcid.org/0009-0000-2917-0263
anitaignasiak@interia.pl

Michał Cisowski
Central Clinical Hospital of the Medical University of Łódź
ul. Pomorska 251, 92-213 Łódź, Poland
https://orcid.org/0009-0005-3977-8244
michal.cisowski@stud.umed.lodz.pl

Maria Dąbrowska
J. Struś Multispecialist Municipal Hospital in Poznań
ul. Szwajcarska 3, 61-285 Poznań
https://orcid.org/0009-0005-6115-0701
marysia-dabrowska1@wp.pl

Kacper Rychlica
The Nicolaus Copernicus Provincial Multispecialty Center for Oncology and Traumatology in Łódź
ul. Pabianicka 62 93-513 Łódź
https://orcid.org/0009-0003-6103-6234
kacperychlica@gmail.com

Jolanta Cholewińska-Rychlica
The Nicolaus Copernicus Provincial Multispecialty Center for Oncology and Traumatology in Łódź
ul. Pabianicka 62 93-513 Łódź
https://orcid.org/0009-0002-8254-4994
jcholewinska224@gmail.com

Paulina Madura
Independent Public Healthcare Institution MSWiA in Łódź
ul. Północna 42, 91-425 Łódź
https://orcid.org/0009-0008-2141-5279
paulinamadura00@gmail.com

Daria Mrozik-Gałecka
Independent Public Healthcare Institution MSWiA in Łódź
ul. Północna 42, 91-425 Łódź
https://orcid.org/0009-0002-2853-5560
daria.mrozik99@gmail.com

Corresponding author:
Konrad Gawin, konrad.gawin1@icloud.com

Abstract
Chronic obstructive pulmonary disease (COPD) is a heterogeneous respiratory disorder whose diagnosis and differentiation from asthma remain challenging. The aim of this review was to present current biomarkers used in the diagnosis of COPD and to highlight promising directions for future research. A literature analysis covering the years 2020–2025 included biomarkers assessed in blood, urine, sputum, as well as genetic and molecular markers. The findings indicate that peripheral blood eosinophil counts and the neutrophil-to-lymphocyte ratio (NLR) are useful in predicting the risk of exacerbations and response to therapy. Serum proteomic and metabolomic analyses, together with urinary biomarkers, enable non-invasive patient phenotyping and early detection of exacerbations. Genetic and transcriptomic studies have identified key genes and inflammatory pathways relevant to disease pathogenesis, while the composition of the airway microbiome correlates with symptom severity and lung function. Integration of molecular, immunological, and microbiological data allows for precise patient phenotyping, supports personalized therapeutic strategies, and improves clinical outcomes.

Biomarkers are becoming an important source of information in the differential diagnosis of COPD and asthma–COPD overlap (ACO), as well as in targeted treatment approaches.

Aim

The aim of this study is to review and summarize current evidence on novel biomarkers of chronic obstructive airway diseases and to assess their impact on differential diagnosis, therapeutic strategies, and prognosis, with particular emphasis on chronic obstructive pulmonary disease (COPD).

Material and methods

A literature review of studies published between 2020 and 2025 was conducted using the PubMed database with predefined keywords. Publications not available in English and studies involving exclusively pediatric populations were excluded. From the remaining articles, data regarding novel biomarkers used in the diagnosis of obstructive lung diseases, the characteristics of the studied populations, and key findings related to differential diagnosis were extracted and subsequently synthesized thematically.

Results

The literature analysis demonstrated that inflammatory biomarkers, including peripheral blood eosinophils and neutrophils, the neutrophil-to-lymphocyte ratio (NLR), C-reactive protein (CRP), as well as metabolic and proteomic markers in serum and urine, may support early diagnosis, assessment of exacerbations, and evaluation of the risk of COPD progression. Data from molecular studies indicate that dysregulated gene expression (e.g., PRPF19, PPIB, EXPH5) and epigenetic mechanisms may serve as potential diagnostic and therapeutic biomarkers. Furthermore, analysis of the airway microbiome contributes to a better understanding of disease pathogenesis and facilitates the identification of novel diagnostic and therapeutic targets. Urinary-based studies have shown that concentrations of biomarkers such as glyphosate and 11-dehydro-thromboxane B2 correlate with symptom severity and COPD risk, highlighting the importance of environmental exposure and platelet metabolism in the course of the disease.

Conclusion

Novel molecular, immunological, and metabolic biomarkers have the potential to support differential diagnosis, prognostication, and personalized therapy in COPD. Integration of data from multiple sources (blood, urine, saliva, microbiome, and genetic analyses), along with further validation studies, is essential for their implementation into routine clinical practice.

Key words

COPD (chronic obstructive pulmonary disease), Biomarkers, Asthma-COPD Overlap (ACO), Eosinophils, Sputum analysis.

Introduction

Chronic respiratory diseases represent one of the most serious health challenges of contemporary society. Since 1990, the number of patients affected by these conditions has increased by nearly 40%, with the most pronounced growth observed in countries with higher levels of economic development. This phenomenon is associated, among other factors, with long-term exposure to air pollution and the widespread prevalence of unfavorable health

behaviors, including tobacco smoking and the use of electronic cigarettes. (GBD Chronic Respiratory Disease Collaborators, 2020)[1](Adeloye et al 2019)[2] Chronic respiratory diseases currently constitute the third leading cause of death worldwide, surpassed only by cardiovascular diseases and malignancies. Among these conditions, chronic obstructive pulmonary disease (COPD) and asthma play a dominant role.(Adeloye et al 2015)[3]

Chronic obstructive pulmonary disease is a complex and heterogeneous respiratory disorder characterized clinically by persistent respiratory symptoms such as chronic cough, dyspnea, and sputum production. The disease is defined by persistent and progressively worsening airflow limitation resulting from pathological changes within the bronchi and bronchioles, including chronic inflammation and excessive mucus production, as well as from destruction of the lung parenchyma caused by long-term exposure to harmful agents.[Celi et al, 2022][4]

According to data from the World Health Organization, chronic obstructive pulmonary disease is responsible for approximately 3.5 million deaths annually worldwide, accounting for nearly 5% of all global deaths. Importantly, almost 90% of COPD-related deaths occur in low- and middle-income countries.[World Health Organization, 2023][5]

The development of the disease results from multifactorial interactions between individual susceptibility and environmental exposure. Tobacco smoking remains the most significant risk factor, accounting for nearly 80% of COPD cases. However, accumulating evidence indicates that long-term exposure to air pollution also plays a substantial role in disease pathogenesis. The fundamental mechanism underlying COPD is a chronic inflammatory process induced by harmful environmental agents. The inflammatory response involves, among others, Tc1, Th1, and Th17 lymphocytes as well as ILC3 cells, and may persist even after removal of the triggering factor.(Szczeklik & Gajewski, 2024)[6]

The pathogenesis of COPD is multifactorial and involves the coexistence of two key processes: pulmonary emphysema and obstruction of the bronchi and bronchioles. Two principal pathophysiological mechanisms contribute to lung parenchymal damage: excessive proteolytic activity and increased oxidative stress. These processes initiate a cascade of changes, including increased mucus production and impaired mucociliary clearance, leading to airflow limitation, lung hyperinflation, development of emphysema, impaired gas exchange, and ultimately pulmonary hypertension and cor pulmonale. Excessive mucus secretion manifests clinically as chronic productive cough, one of the hallmark symptoms of the disease. Narrowing of the small airways results from both bronchial and bronchiolar obstruction and increased lung compliance secondary to emphysematous changes. Direct causes of obstruction include smooth muscle contraction, chronic bronchiolitis with subsequent peribronchial fibrosis leading to permanent airway narrowing, and airway filling with inflammatory cells and exudate. Air trapping arises as a consequence of impaired expiration, during which structurally altered bronchioles collapse, preventing effective air evacuation from the lungs. Pulmonary emphysema is characterized by pathological enlargement of airspaces distal to the terminal bronchioles, resulting in the formation of large alveolar spaces, loss of functional respiratory surface area, and significant impairment of gas exchange.(Szczeklik & Gajewski, 2024)[6](Barnes et al. 2009)[7](GOLD, 2025)[8] Moreover, COPD frequently coexists with cardiovascular diseases, which influences

therapeutic decision-making and may require concurrent management of both conditions to improve clinical outcomes. Comprehensive assessment of COPD phenotypes, immunological markers, and comorbidities may support a personalized therapeutic approach and provide better insight into disease pathophysiology and its impact on the cardiovascular system.(Schivo et.al 2017)[9]

Spirometry is regarded as the most reliable and objective method for assessing the degree of airflow obstruction. It is a non-invasive, widely available examination with relatively low cost and constitutes the cornerstone of diagnostic evaluation for obstructive lung diseases, enabling quantitative assessment of ventilatory function and detection of obstructive abnormalities (GOLD, 2025) [8]. Spirometry is essential in all patients with suspected COPD or asthma, both at the stage of diagnosis and during disease monitoring. In COPD diagnostics, spirometry is routinely performed after administration of a bronchodilator, most commonly 400 µg of salbutamol. A diagnosis of COPD can be established when the post-bronchodilator FEV_1/FVC ratio remains below 0.70, indicating persistent airflow obstruction. The diagnosis also requires the presence of compatible clinical symptoms, such as chronic cough or exertional dyspnea. These diagnostic criteria are defined in the Global Initiative for Chronic Obstructive Lung Disease guidelines (Szczeklik i Gajewski, 2024) [6]; (GOLD, 2025) [8].

Spirometry remains one of the most classical, yet also the most effective and objective diagnostic methods for chronic obstructive pulmonary disease. However, this review places particular emphasis on emerging and alternative diagnostic strategies, including the detection of COPD, its exacerbations and remission phases, and the evaluation of their relevance for therapeutic decision-making and prognosis. Contemporary medicine increasingly focuses on molecular and genetic diagnostics, including the identification of substances with potential biomarker relevance across different clinical states of COPD. Phenotyping patients based on shared clinical or molecular characteristics enables improved tailoring of therapy, and current COPD guidelines increasingly emphasize the concept of “treatable traits” — characteristics for which evidence-based, targeted interventions exist. Advances in lung imaging, physiological measurements, omics technologies, and biomarker research have enhanced understanding of disease heterogeneity, while integration of these data allows more precise phenotype classification and personalized management.(Christenson, 2023)[10]

Phenotypic classification enables identification of patient subgroups, such as those with predominant emphysema or frequent exacerbations, whereas endotypes — defined by specific biological mechanisms such as neutrophilic or eosinophilic inflammation or α1-antitrypsin deficiency — more accurately predict treatment response. Biomarkers, including blood eosinophil counts, CRP levels, and sputum transcriptomic analyses, facilitate patient stratification and guide targeted therapies, including inhaled corticosteroids and biological agents. The “treatable traits” concept additionally incorporates modifiable factors such as comorbidities, psychosocial determinants, and exacerbation triggers. Despite advances in diagnostics and therapy, challenges remain regarding biomarker validation, standardization of phenotype definitions, and translation of research findings into routine clinical practice. The future of COPD management is likely to involve early detection of pre-COPD states, integration

of multi-omics data, dynamic phenotyping supported by machine learning, and clinical trials evaluating interventions tailored to individual disease mechanisms.(Xie et al. 2025)[11]

Blood/plasma

A minimally invasive source of biomarkers is blood, which is widely used for research purposes because peripheral blood samples accurately reflect metabolic changes occurring in the body and can be collected in a low-invasive and easily repeatable manner. Respiratory system cells, at the time of injury or cell death, release part of their intracellular contents into the bloodstream, making serum one of the most important sources of information. The detection of various substances in blood or changes in the ratio of one molecule to another may represent the first manifestation of obstructive lung disease, disease exacerbation, progression, or remission.(Fang et al. 2023)[12]

Studies indicate that serum analysis enables not only early detection of pathological changes but also identification of COPD subtypes with distinct clinical courses. In a study by Zili Zhang et al. (2023), significant clinical and molecular differences were demonstrated between patients with advanced chronic obstructive pulmonary disease (COPD) and healthy individuals, including poorer ventilatory parameters (FEV_1, FEV_1/FVC), a higher prevalence of cardiovascular diseases, reduced mean platelet volume (MPV), and an increased proportion of monocytes. Serum proteomic and metabolomic analyses revealed pronounced disturbances in inflammatory and immune responses, activation of metabolic pathways (glycolysis, purine metabolism, carbon metabolism), and alterations in extracellular matrix proteins and the coagulation system. Based on proteomic profiles, three COPD subtypes were identified that differed in clinical course and comorbidities, including a form without additional respiratory diseases, COPD with bronchiectasis, and COPD coexisting with metabolic syndrome, with patients with COPD and bronchiectasis exhibiting the lowest FEV_1 values. An integrated multi-omics approach enabled the development of diagnostic panels with very high accuracy in identifying advanced COPD, with combinations of selected metabolites and proteins, including palmitoylethanolamide, theophylline, hypoxanthine, and cadherin 5 (CDH5), showing the highest diagnostic value (sensitivity 0.94; specificity 0.95), whereas these markers did not demonstrate significant differences in patients with mild and moderate disease. (Zhang et al. 2023)[13]

At the same time, research into the pathogenic mechanisms of COPD highlights the role of immune system cells, particularly neutrophils. The review by Shi-Xia Liao et al. (2025) clearly indicates that polymorphonuclear neutrophils play a key role in COPD pathogenesis, constituting the dominant population of inflammatory cells in the airways of affected patients. Excessive neutrophil activation, including phagocytosis, degranulation, and the formation of neutrophil extracellular traps (NETs), significantly contributes to respiratory epithelial damage, emphysema development, and mucus hypersecretion.(Shi-Xia Liao et al 2025)[14]

Similarly, a study by Huanga et al. (2024) demonstrated that patients with COPD exhibit significant activation of inflammatory pathways, particularly inflammatory responses and TNF-

α/NF-κB signaling, with marked intensification of these processes in neutrophils and dendritic cells. Cell differentiation trajectory analyses indicated delayed neutrophil maturation in COPD, with the most advanced differentiation stages associated with the strongest immune activation. Concurrently, increased neutrophil infiltration in lung tissue was confirmed, along with significant interactions between neutrophils and other immune cell populations. Integration of differential gene expression analyses, co-expression networks, and Mendelian randomization enabled identification of key genes causally associated with COPD, including NAMPT and PTGS2, whose overexpression was negatively correlated with lung function parameters. These findings, validated in peripheral blood leukocytes, underscore the central role of neutrophilic inflammation in COPD progression and highlight selected genes as potential biomarkers of disease activity and therapeutic targets.[Huanga et al. 2024)[15]

The prognostic significance of neutrophils as biomarkers is further supported by a meta-analysis by Li Fang et al. (2025), encompassing 24 studies and a total of 18 597 patients with COPD. This analysis demonstrated a significant association between elevated neutrophil-to-lymphocyte ratio (NLR) and increased all-cause mortality risk. Patients with high NLR values exhibited a significantly greater risk of death compared with those with lower NLR, both in categorical and continuous analyses, and deceased individuals had significantly higher NLR values than survivors. Subgroup analyses indicated that the prognostic value of NLR was particularly pronounced in patients with COPD exacerbations, while heterogeneity may have resulted from differences in study design and timing of NLR measurement. These findings confirm the potential utility of NLR as a simple and accessible prognostic biomarker in COPD.(Fang et al. 2025)[16]

Concurrently, studies on COPD emphasize the role of eosinophils, highlighting the complex nature of inflammation in this disease. A review by Papaporfyriou et al. (2022) indicates that a significant eosinophilic component is present in a subset of COPD patients, observed both during exacerbations and in the stable phase of the disease, challenging the exclusively neutrophilic inflammatory model. The authors emphasize that increased peripheral blood eosinophil counts correlate with distinct clinical features of COPD and response to anti-inflammatory treatment, particularly inhaled corticosteroids and biological therapies. Peripheral blood eosinophilia currently appears to be the most useful prognostic and theranostic biomarker in COPD, enabling a more personalized therapeutic approach.(Papaporfyriou et al. 2022)[17]

Clinical studies provide specific thresholds and predictive values for eosinophilia. In a study by Kang et al. (2021), a significant positive association was demonstrated between peripheral blood eosinophil counts during stable COPD and eosinophil levels during disease exacerbations. The analysis showed that an optimal cutoff value for eosinophil counts in stable COPD for predicting eosinophilic exacerbations was 300 cells/μl, regardless of whether eosinophilic AECOPD was defined as ≥2% eosinophils or ≥300 cells/μl. Although the sensitivity of this threshold was moderate, it exhibited high specificity, indicating potential clinical utility in identifying patients predisposed to eosinophilic COPD exacerbations and in personalizing anti-inflammatory treatment.(Kang et al. 2021)[18]

Similar conclusions were drawn from a study by Zhanga et al. (2020), which assessed the association between peripheral blood eosinophilia, measured both at hospitalization for COPD exacerbation and its long-term stability, and all-cause mortality risk. It was shown that patients hospitalized for AECOPD with elevated eosinophil levels (≥150 cells/μL) had significantly lower all-cause mortality compared with patients without eosinophilia. Long-term analysis further demonstrated that patients with persistent, dominant eosinophilia across consecutive hospitalizations had a markedly lower mortality risk than those with infrequent eosinophilia, whereas this association was weaker and not statistically significant in patients with intermittent eosinophilia. These results suggest that peripheral blood eosinophilia, particularly when stable, may serve as a useful prognostic biomarker in patients hospitalized for COPD exacerbations.(Zhanga et al. 2020)[19]

In a meta-analysis by Liu et al. (2023), including 15 methodologically high-quality studies, the prognostic significance of peripheral blood eosinophilia was evaluated in patients hospitalized for COPD exacerbations. Patients with eosinophilic AECOPD had significantly lower all-cause mortality compared with those without eosinophilia, as well as shorter hospital stays. At the same time, this group exhibited a slightly but statistically significantly higher rate of hospital readmissions. No differences were observed between groups in the frequency of subsequent hospitalizations or the need for invasive mechanical ventilation. These findings confirm the potential usefulness of blood eosinophil levels as a prognostic biomarker in patients with AECOPD.(Liu et al. 2023)[20]

The role of eosinophilia as a biomarker of recurrent COPD exacerbation risk is further supported by prospective studies. In a study by Kiani et al. (2023), involving 973 newly diagnosed COPD patients followed for one year, a significant positive association was observed between peripheral blood eosinophil counts at diagnosis and exacerbation frequency. Higher eosinophil values correlated with a greater annual number of AECOPD events, with a threshold of approximately 800 cells/μl demonstrating the best predictive ability for exacerbation occurrence, combining high sensitivity and specificity. Multivariate analysis showed that both elevated eosinophil counts and higher disease severity according to the GOLD classification were independent risk factors for frequent exacerbations, whereas no differences were observed between eosinophilic and non-eosinophilic groups regarding ICU admission, invasive ventilation, or mortality. These results confirm the importance of eosinophilia as a biomarker of recurrent COPD exacerbation risk.(Kiani et al. 2023)[21]

Similarly, a multicenter prospective study by Pu et al. (2023), involving more than 12 800 patients hospitalized for COPD exacerbations, assessed the prognostic significance of peripheral blood eosinophil percentage. Patients without eosinophilia (< 2% eosinophils) had significantly higher in-hospital mortality in the overall population and in subgroups with pneumonia and respiratory failure, whereas this association was not observed in patients treated in the intensive care unit. Regardless of the subgroup analyzed, the non-eosinophilic AECOPD phenotype was associated with more frequent need for invasive mechanical ventilation, ICU admission, and longer hospital stays, as well as—paradoxically—more frequent use of systemic glucocorticoids. These findings indicate that blood eosinophil levels measured at admission

may serve as a useful prognostic biomarker in most patients with AECOPD, except for those requiring ICU treatment.(Pu et al. 2023)[22]

Finally, the stability of the eosinophilic phenotype across successive exacerbations was evaluated in a study by Citgez et al. (2021). The concordance of the eosinophilic phenotype between two consecutive exacerbations ranged from 70–85%, depending on the cutoff used (absolute eosinophil count or percentage). At the same time, only 34–45% of patients with eosinophilia during the first exacerbation maintained this phenotype during the subsequent episode, while 9–21% of initially non-eosinophilic patients developed eosinophilia. These results indicate significant variability in eosinophil counts during successive severe COPD exacerbations and emphasize the need for eosinophil measurement at each new AECOPD episode, particularly in the context of therapeutic decision-making.(Citgez et al. 2021)[23]

Measurement of markers in peripheral blood and serum constitutes a valuable, minimally invasive tool in the diagnosis and monitoring of COPD, as it reflects both systemic and local inflammatory and metabolic processes. In addition to classical inflammatory indices, such as the neutrophil-to-lymphocyte ratio (NLR) and eosinophil counts, whose elevated levels correlate with disease severity, exacerbation risk, and poorer clinical outcomes, proteomic and metabolomic studies identify additional potential markers. These include alterations in immune and coagulation system proteins and specific metabolites (e.g., palmitoylethanolamide, hypoxanthine, theophylline, cadherin 5), which enable differentiation of COPD subtypes with distinct clinical courses. Genetic and epigenetic analyses have identified dysregulated expression of genes related to immune responses and cellular metabolism, which may further support diagnostic and prognostic assessment. The diversity of these markers and their associations with clinical features of COPD underscore their potential role in therapy individualization and improvement of prognostic stratification in affected patients.

Neutrophil–lymphocyte ratio (NLR)

The analysis of inflammatory indices in peripheral blood is becoming increasingly important in the context of predicting clinical outcomes in patients with acute exacerbations of chronic obstructive pulmonary disease (AECOPD). In a systematic review by Zinellu et al. (2022), 18 studies examining the association between the neutrophil-to-lymphocyte ratio (NLR) and clinical outcomes in patients with AECOPD, as well as 10 studies assessing the platelet-to-lymphocyte ratio (PLR), were analyzed. The results indicated that NLR was significantly higher in patients who died during hospitalization and correlated with C-reactive protein (CRP) levels and length of hospital stay. In some studies, NLR was also associated with the risk of transfer to the intensive care unit and the need for mechanical ventilation, and its values demonstrated high predictive accuracy in ROC analyses. Combining NLR with PLR and CRP further improved the prediction of adverse outcomes. In contrast, results regarding PLR were less consistent—some studies demonstrated an independent association with mortality risk or pulmonary hypertension, whereas others did not confirm statistical significance. Overall, NLR appears to be a more stable and reliable prognostic marker in AECOPD, while PLR may serve a complementary role, particularly when combined with other inflammatory markers.(Zinellu et al. 2022)[24]

These observations were confirmed in a retrospective study by Yao et al. (2017), which included 303 patients hospitalized for AECOPD. Both the neutrophil-to-lymphocyte ratio (NLR) and the platelet-to-lymphocyte ratio (PLR) were significantly higher in patients who died during hospitalization compared with those who survived the exacerbation. NLR was positively correlated with C-reactive protein levels, and its prognostic value for in-hospital mortality was high, with a cut-off value of approximately 6.2, demonstrating good sensitivity and specificity and the highest area under the ROC curve. PLR showed a weaker but still significant predictive ability, whereas combined analysis of NLR, PLR, and CRP further improved the accuracy of mortality risk assessment. These findings indicate that NLR, in particular, may serve as a simple and readily available prognostic biomarker in patients with AECOPD.(Yao et al. 2017)[25]

Further studies emphasize the importance not only of absolute NLR values but also of their dynamic changes during hospitalization. In a retrospective study by Jiang et al. (2024), including 841 patients hospitalized for AECOPD, the stability of NLR and its association with clinical outcomes were evaluated. Patients were classified into four groups based on NLR trajectories: persistently high, increasing, decreasing, and persistently low. The persistently high NLR group exhibited the poorest in-hospital outcomes, including more frequent use of mechanical ventilation, transfers to the intensive care unit, longer hospital stays, and higher treatment costs. In contrast, patients with persistently low NLR had a significantly lower risk of death (HR: 0.13) and more favorable clinical outcomes, while the decreasing NLR group also demonstrated a significantly reduced mortality risk (HR: 0.40). These findings suggest that NLR stability may serve as a useful prognostic biomarker for identifying patients with AECOPD at increased risk of adverse in-hospital outcomes.(Jiang et al. 2024)[26]

Analogous observations were confirmed in a study by Vu-Hoai et al. (2024), which included 287 patients hospitalized for AECOPD. The neutrophil-to-lymphocyte ratio (NLR) was identified as a significant predictor of adverse clinical outcomes, defined as the need for mechanical ventilation, admission to the intensive care unit, or in-hospital death. Multivariate logistic regression analysis demonstrated that elevated NLR (cut-off 11.0) significantly increased the risk of adverse events (OR = 13.9, 95% CI: 6.3–30.7, $P < 0.001$), with a sensitivity of 80.0% and specificity of 77.7%, and an area under the ROC curve of 0.877. Reduced level of consciousness at admission was also predictive of poorer outcomes (OR = 0.08, 95% CI: 0.02–0.38, $P = 0.001$). This study confirms that NLR is a simple, routine, and cost-effective prognostic marker in AECOPD and suggests the need for further research on its dynamic changes in monitoring treatment response.(Vu-Hoai et al. 2024)[27]

The prognostic value of NLR has also been confirmed in the context of mid-term mortality. In a retrospective study by Feng et al. (2023), involving 503 patients hospitalized for AECOPD, the neutrophil-to-lymphocyte ratio (NLR) was identified as a significant independent predictor of 90-day mortality. Multivariate analysis identified independent risk factors for death within 90 days, including age >72 years (OR = 14.8), NLR >14.17 (OR = 9.6), EOS <0.15% (OR = 8.6), and BNP >2840 ng/L (OR = 5.3). NLR was the best predictive marker for 90-day mortality (AUC = 0.802), with a sensitivity of 76.7% and specificity of 88.9% at a cut-off value of 14.17. In contrast, NLR demonstrated limited usefulness in predicting recurrent exacerbations or

readmissions within 90 days (AUC <0.6), while EOS slightly better predicted readmission risk. These results suggest that NLR is a simple and useful prognostic biomarker for mortality risk in hospitalized patients with AECOPD within 90 days after discharge.(Feng et al. 2023)[28]

In summary, study findings consistently indicate that the neutrophil-to-lymphocyte ratio (NLR) is a simple, readily available, and reliable prognostic biomarker in acute exacerbations of COPD. Its relevance encompasses both short-term mortality and major clinical events during hospitalization. PLR may serve a complementary role, particularly when combined with other inflammatory markers such as CRP. Additionally, the dynamic changes and stability of NLR during hospitalization appear to be important for predicting adverse outcomes, underscoring the potential of this index in monitoring treatment response and individualizing care in patients with AECOPD.

Urine

Urine is increasingly recognized as a valuable source of biomarkers due to the possibility of non-invasive and continuous sample collection, which represents a significant advantage over other biological materials. Urinary proteomic studies have demonstrated that urine can provide sensitive indicators of respiratory system diseases, including lung cancer, pulmonary fibrosis, ventilator-induced lung injury, and acute respiratory distress syndrome (ARDS). These findings indicate that urine represents a promising medium for biomarker discovery in lung diseases and may support the development of diagnostic and monitoring strategies for these conditions, including in the course of chronic obstructive pulmonary disease (COPD). (Fang et al. 2023)[29]

In the context of COPD, particular attention has been paid to the relationship between urinary markers and the severity of clinical symptoms. In a study by Fawzy et al. (2023), the association between platelet activation and respiratory symptoms was evaluated in 169 former smokers with spirometry-confirmed moderate-to-severe stable COPD. Urinary concentrations of the thromboxane A2 metabolite 11-dehydro-thromboxane B2 (11dTxB2), as well as plasma levels of soluble CD40L and P-selectin, were measured repeatedly over a period of 6–9 months. The results showed that a doubling of urinary 11dTxB2 concentration was associated with a significant worsening of respiratory symptoms (higher scores on the COPD Assessment Test and the Ease of Cough and Sputum Clearance Questionnaire), poorer health status (Clinical COPD Questionnaire), and reduced quality of life (St George's Respiratory Questionnaire). No statistically significant associations were observed between sCD40L or sP-selectin and clinical outcomes. Moreover, a history of cardiovascular disease, subclinical coronary artery disease, use of antiplatelet therapy, or COPD severity did not significantly modify the observed association between 11dTxB2 and symptoms. The authors concluded that urinary 11dTxB2 may serve as a potential biomarker of increased symptom burden and functional impairment in patients with COPD, highlighting the role of platelet activation in the pathophysiology of the disease. (Fawzy et al. 2023)[30]

These observations are complemented by studies focusing on metabolic disturbances associated with COPD, assessed in various body fluids, including urine. In a study by Kim et al. (2022), metabolic mechanisms and potential biomarkers of COPD progression were analyzed in a

PPE/LPS-induced exacerbation model, incorporating metabolic changes in the lungs, plasma, and urine, as well as their correlations with lung morphology and function. The authors demonstrated that metabolic reprogramming occurs in the lungs in response to disease development. In plasma, combinations of phenylalanine, 3-methylhistidine, and polyunsaturated fatty acids, as well as a novel α-aminobutyric acid/histidine index, were identified as potential biomarkers. In urine, succinic acid, isocitric acid, and pyruvic acid emerged as potential biomarkers. These results suggest that metabolites detected in plasma and urine reflect COPD-related metabolic alterations in the lungs, which may support diagnosis, assessment of disease severity, and the identification of therapeutic targets. (Kim et al. 2022)[31]

An important step toward the practical implementation of urinary biomarkers in routine COPD care involves studies evaluating their utility in detecting and predicting exacerbations. In a study by Yousuf et al. (2025), a panel of 10 urinary biomarkers (NGAL, TIMP1, CRP, fibrinogen, CC16, fMLP, TIMP2, A1AT, B2M, and MMP8) was identified that enables discrimination between stable COPD and exacerbation states. In an exploratory analysis conducted in 55 patients, the panel demonstrated high diagnostic performance (AUC 0.84; 95% CI 0.76–0.92; $p < 0.01$), which was confirmed in a prospective validation study including 105 patients (AUC 0.81; 95% CI 0.70–0.92; $p < 0.01$). Furthermore, using an artificial neural network (ANN) developed based on biomarker data from 85 participants, COPD exacerbations could be predicted with a median lead time of 7 days (IQR 5–9 days) before the onset of clinical symptoms, within a 13-day observation window (AUC 0.89; 95% CI 0.89–0.90). These findings suggest that daily monitoring of urinary biomarkers may represent a non-invasive and sensitive method for early detection of COPD exacerbations and prediction of exacerbation risk, potentially enabling earlier therapeutic interventions and more effective disease management. (Yousuf et al. 2025)[32]

In addition to biomarkers reflecting inflammatory and metabolic processes, urine analysis may also provide information on environmental exposures that can influence the risk of developing COPD. A study by Shi et al. (2025) demonstrated a significant association between higher urinary glyphosate concentrations and an increased risk of chronic obstructive pulmonary disease. Analysis of 2,588 adult participants showed that each increase in the natural logarithm of urinary glyphosate concentration was associated with a 35% increase in COPD risk (OR 1.35; 95% CI 1.01–1.82; $p = 0.043$). Subgroup analyses revealed consistent associations across all demographic groups, with the strongest effects observed among current smokers and women. The results were robust to sensitivity analyses and after exclusion of individuals with chronic kidney disease. These data suggest that environmental exposure to glyphosate may represent an important risk factor for COPD, underscoring the need for further studies addressing the combined effects of multiple environmental pollutants. (Shi et al. 2025)[33]

Urine represents a promising, non-invasive source of biomarkers for the diagnosis and monitoring of chronic obstructive pulmonary disease. Available evidence indicates that both individual markers, such as 11-dehydro-thromboxane B2, and complex proteomic and metabolic profiles can reflect symptom severity, functional status, and the risk of COPD exacerbations. Moreover, analysis of urinary metabolites and inflammatory biomarkers enables

assessment of pulmonary pathophysiological processes and identification of environmental factors that increase disease risk, such as glyphosate exposure. These findings highlight the potential of urine as a tool to support early diagnosis, prognostic assessment, and personalization of therapeutic management in patients with COPD.

Sputum microbiome and *Pseudomonas aeruginosa*

Increasing evidence indicates that the respiratory tract microbiome plays a key role in the pathogenesis of chronic obstructive pulmonary disease (COPD) and in the mechanisms leading to its exacerbations. In a review by Liu et al. (2021), current data on COPD and acute exacerbations of the disease (AECOPD) were presented, emphasizing the importance of pulmonary microbiota dysbiosis in initiating and sustaining chronic inflammation. The authors highlighted that alterations in lung microbiome composition can exacerbate inflammatory processes, contributing to increased dyspnea, cough, and sputum production. Analysis of available studies suggests that microbiota imbalance participates in mechanisms triggering COPD exacerbations, opening the perspective for using the microbiome as a potential diagnostic and therapeutic target. Integration of findings from different studies allows for a better understanding of disease course and mechanisms and provides directions for future therapeutic strategies.(Liu et al. 2021)[34]

This issue has been further explored in studies analyzing the sputum microbiome across different disease phases. In the study by Su et al. (2022), significant differences in the composition and function of the sputum microbiome were observed between patients during AECOPD, stable disease, convalescence, and healthy controls. Patients with AECOPD exhibited a marked reduction in bacterial diversity compared with stable COPD and control groups, with a dominance of Proteobacteria and a relative increase in Actinobacteria at the expense of Firmicutes and Bacteroidetes. Haemophilus was identified as a characteristic taxon associated with exacerbations, while functional analysis revealed enrichment of pathways related to membrane transport and cellular signal transduction. Furthermore, associations were demonstrated between microbiome composition and clinical parameters, including a positive correlation of Veillonella with lung function and Staphylococcus with inflammation severity. These results indicate that respiratory dysbiosis may play a significant role in the pathogenesis and progression of AECOPD and may serve as a potential source of biomarkers useful for the diagnosis and assessment of COPD exacerbations.(Su et al. 2022)[35]

The importance of microbiota diversity and its association with disease severity has also been confirmed in analyses of lower respiratory tract specimens. In the study by Bahetjan et al. (2025), bronchoalveolar lavage fluid (BALF) microbiota from 70 patients with AECOPD was analyzed, comparing groups with mild versus severe airflow limitation and patients with frequent versus infrequent exacerbations. Patients with severe airflow limitation exhibited significantly reduced microbial diversity as well as lower species abundance and richness. The most frequently identified microorganisms were Streptococcus, Prevotella, Veillonella, Gram-negative rods, and Rothia, while Aspergillus predominated among fungi and Lymphocryptovirus among viruses. Analysis revealed specific patterns of taxon enrichment depending on the degree of obstruction and exacerbation frequency, highlighting the critical

role of the microbiota in AECOPD pathogenesis and suggesting potential therapeutic targets.(Bahetjan et al. 2025)[36]

Similar relationships have been observed during the stable phase of the disease. In a study by Yang et al. (2019), patients with COPD at high risk of exacerbations exhibited significantly reduced sputum bacterial diversity compared with patients at low risk of exacerbations. These changes affected both taxonomic and functional microbiome composition, and correlation analysis demonstrated associations between the abundance of specific bacteria and lung function parameters. These findings suggest that sputum microbiome dysbiosis is linked to disease phenotype and increased exacerbation risk, highlighting the potential of the microbiome as a modifiable clinical factor.(Yang et al. 2019)[37]

Of particular interest in the context of COPD exacerbations is Pseudomonas aeruginosa infection, which is associated with more severe disease and poorer prognosis. In a study by Lin et al. (2025), a comprehensive proteomic and metabolomic analysis of BALF and serum from patients with P. aeruginosa-induced pneumonia demonstrated significant activation of neutrophil extracellular trap (NETs) pathways and oxidative stress. Integration of local and systemic data revealed host metabolic reprogramming as well as potential diagnostic biomarkers and therapeutic targets.(Lin et al. 2025)[38]

These observations were complemented by a multi-omics study on AECOPD with P. aeruginosa infection, combining proteomic, transcriptomic analyses, and biomarker validation. Key proteins associated with NETs and oxidative stress were identified, confirming their high diagnostic value and potential therapeutic relevance.(Lin et al. 2025)[39]

Mechanisms of host interaction with P. aeruginosa have also been detailed by Verceles et al. (2021), who demonstrated that bacterial flagellin induces cleavage of the extracellular domain of MUC1 (MUC1-ED) in airway epithelial cells. The released desialylated MUC1-ED exhibited properties that limit bacterial motility, biofilm formation, and adhesion, while simultaneously enhancing neutrophil phagocytosis. These findings suggest that MUC1-ED may serve as a novel biomarker and a component of host defense in P. aeruginosa infections.(Verceles et al. 2021)[40]

Overall, available data indicate that respiratory microbiome disturbances play a significant role in COPD pathogenesis and in mechanisms leading to acute exacerbations. Reduced bacterial diversity and dominance of specific taxa, particularly Proteobacteria, correlate with more severe disease, poorer lung function, and increased exacerbation risk. Pseudomonas aeruginosa infections are of particular importance, being associated with enhanced oxidative stress, NETs activation, and worse clinical outcomes. Studies integrating microbiome, proteomic, and metabolomic analyses suggest the possibility of identifying specific diagnostic and prognostic biomarkers, as well as potential therapeutic targets. Microbial characterization of sputum and lower airway specimens may in the future support a more personalized approach to the diagnosis and management of patients with COPD.

Genetics

There is no doubt that the future of modern medicine is closely linked to the dynamic development of genetic and molecular research. Both the diagnosis and treatment of diseases increasingly rely on precise identification, modulation, and suppression of specific genes and molecular pathways. Currently, the scientific literature contains numerous publications regarding gene expression disorders, epigenetic mechanisms, and deregulation of transcriptional processes in the course of chronic obstructive pulmonary disease (COPD). Despite high costs and significant technical and interpretative challenges, this direction represents a promising and prospective pathway for the advancement of personalized medicine.[Zhang et al. 2023][41]

In this context, studies integrating different levels of genetic regulation are of particular importance. In a study by Zhao et al. (2025), an integrated analysis of peripheral blood DNA methylome and transcriptomic data allowed for the identification of numerous epigenetic and expression disturbances associated with COPD, encompassing over 10,000 genes with differential methylation and 646 genes with altered expression. Combining gene co-expression network analysis with protein–protein interaction networks identified five genes critical for disease pathogenesis (PPIB, HSPA2, PRPF19, FKBP10, DOHH). Expression of PRPF19, PPIB, FKBP10, and DOHH was significantly upregulated in COPD patients, whereas HSPA2 exhibited decreased expression. Mendelian randomization analysis indicated a potential causal relationship between PRPF19 and PPIB and COPD development, which was confirmed in validation studies using RT-qPCR, immunohistochemistry, and Western blot, demonstrating overexpression of both genes in peripheral blood and lung tissue of patients. These findings suggest that PRPF19 and PPIB may serve as promising diagnostic biomarkers for COPD and as potential targets for further pathogenic research.[Zhao et al. 2025][42]

Complementary to these observations are analyses focusing on the identification of gene signatures and their associations with immune responses. In the study by Hui Yu et al. (2021), 127 genes with significantly altered expression were identified in COPD patients, of which 83 genes, selected through co-expression network analysis, demonstrated strong associations with disease phenotype and were functionally linked predominantly to interleukin-dependent pathways. LASSO regression allowed the identification of a seven-gene molecular signature (MTHFD2, KANK3, GFPT2, PHLDA1, HS3ST2, FGG, RPS4Y1) with good predictive capacity for assessing COPD risk. Expression of these genes showed significant correlations with infiltration by 18 immune cell populations, indicating a close relationship with the immunological basis of the disease. Additionally, regulatory analysis revealed that the expression of key signature genes is potentially modulated by specific microRNAs and transcription factors, highlighting the complex transcriptional and post-transcriptional mechanisms in COPD pathogenesis and their potential significance as clinical biomarkers.[Hui Yu et al. 2021][43]

Further studies focus on molecular differences between disease stages. In a study by Yuwei Yang et al. (2023), based on integrated GEO transcriptomic datasets, gene expression profiles and immune cell infiltration were compared between early and advanced stages of COPD. A

total of 157 differentially expressed genes were identified, primarily associated with inflammatory processes, cytokine responses, extracellular matrix remodeling, and immune response regulation. Advanced COPD was characterized by activation of chemokine, complement, and coagulation pathways. Machine learning algorithms (LASSO, SVM-RFE) identified EXPH5 as the most promising biomarker of advanced stage, demonstrating significant diagnostic capability in ROC analyses. Notable differences in immune cell infiltration between disease stages were observed, including increased macrophages and B lymphocytes in late-stage COPD and relatively higher percentages of eosinophils and mast cells in early-stage disease. EXPH5 expression positively correlated with eosinophils and mast cells and negatively with γδ T cells and M1 macrophages. Single-cell data analysis confirmed significant downregulation of EXPH5 in advanced COPD, particularly in alveolar type 1 and type 2 (AT1 and AT2) cells, suggesting a potential role for this gene in disease progression and its diagnostic and therapeutic relevance.[Yang et al. 2023][44]

Similarly, the importance of gene expression disturbances in the context of immune responses is underscored by earlier transcriptomic analyses. Zhang et al. (2022) demonstrated significant dysregulation of genes involved in immune response regulation, cellular metabolism, and antigen presentation, highlighting the critical role of inflammatory mechanisms in COPD pathogenesis. Among the analyzed genes, STAU1 and SLC27A3 were identified as potential diagnostic biomarkers for COPD, exhibiting high predictive value and strong correlations with immune cell infiltration profiles. These disturbances included increased proportions of macrophages, memory B cells, and resting mast cells, with concomitant reductions in eosinophils, NK cells, and plasma cells. Expression of STAU1 and SLC27A3 remained closely associated with the intensity of inflammatory response, confirmed in both in vitro and in vivo experimental models. These findings emphasize the significance of the interaction between impaired immune response and molecular alterations in COPD, suggesting the potential application of STAU1 and SLC27A3 as novel diagnostic markers and therapeutic targets.[Zhang et al. 2022][45]

In summary, genetic and transcriptomic studies clearly indicate that COPD is a disease with a complex, multi-level molecular basis, encompassing epigenetic alterations, gene expression deregulation, and abnormal activation of immune and inflammatory pathways. Identification of specific genes and molecular signatures, such as PRPF19, PPIB, EXPH5, STAU1, and SLC27A3, allows increasingly precise differentiation of disease stages, assessment of disease activity, and potential prediction of clinical course. These findings highlight the growing importance of a multi-omics approach in COPD diagnostics and point to genetic biomarkers as promising tools for personalized medicine and future therapeutic interventions.

Computed tomography

With the advancement of imaging techniques and data analysis methods, chest computed tomography (CT) is gaining increasing significance not only as a tool for visual assessment of structural changes but also as a source of quantitative imaging data with high diagnostic value. Radiomics, in particular, plays a crucial role, enabling the extraction and analysis of hundreds of imaging features from entire lungs, which may reflect the complex pathophysiological

processes occurring in COPD.

In the study by Zhou et al. (2024), the potential of quantitative whole-lung CT image analysis as a tool for distinguishing COPD patients from healthy individuals was evaluated. In a cohort of 2,785 patients, a radiomic model based on 18 selected imaging features demonstrated excellent diagnostic performance, achieving AUC values of 0.888 in the training set, 0.874 in internal validation, and 0.846 in an independent validation cohort, significantly outperforming models based solely on clinical variables. Furthermore, the application of a nomogram integrating the radiomic score with age, sex, height, and smoking status improved predictive accuracy, as confirmed by decision curve analysis. These findings suggest that whole-lung CT radiomics represents a promising and precise tool supporting COPD diagnosis in multicenter studies.(Zhou et al. 2024)[46]

Building upon these observations, subsequent analyses focused not only on disease recognition but also on the assessment of disease severity. In Zhou et al. (2024), the utility of quantitative whole-lung CT analysis for evaluating COPD severity was investigated. In a cohort of 1,099 patients, models based on extracted CT imaging features, as well as a nomogram combining these parameters with clinical data, demonstrated superior ability to differentiate mild-to-moderate (GOLD I–II) from severe-to-very severe (GOLD III–IV) disease compared with clinical models alone. The nomogram incorporating age, height, and a composite imaging feature index achieved high diagnostic accuracy, with AUC values of 0.865, 0.851, and 0.781 in the training set, internal validation, and external validation, respectively, and decision curve analysis confirmed its clinical utility. These results highlight that advanced quantitative CT assessment is a valuable tool for determining COPD severity, complementing conventional pulmonary function evaluation with precise characterization of structural changes.(Zhou et al. 2024)[47]

In addition to diagnosis and disease severity stratification, there is growing interest in the use of CT radiomics to identify clinically unstable states, including COPD exacerbations. In the study by Zhou et al. (2025), the utility of quantitative whole-lung CT analysis in recognizing AECOPD was assessed. Analysis of 475 patients demonstrated that a model based on 61 selected CT imaging features, constructed using logistic regression, exhibited very high diagnostic performance in identifying AECOPD, with AUC values of 0.974 in the training set and 0.836 and 0.944 in internal and external validation, respectively, clearly surpassing a model based solely on clinical data. A nomogram combining the imaging feature score with clinical parameters achieved comparable diagnostic accuracy (AUC 0.974, 0.849, 0.957), and decision curve analysis confirmed its high clinical utility. These findings indicate that advanced quantitative assessment of whole-lung CT represents a precise and reliable tool supporting the diagnosis of AECOPD and may have important implications for therapeutic planning.(Zhou et al. 2025)[48]

The role of CT in assessing extrapulmonary complications of COPD, particularly cardiovascular disease, which significantly affects patient prognosis, has also been increasingly emphasized. In the study by Lin et al. (2024), a predictive model based on quantitative analysis of whole-lung CT imaging features combined with selected clinical data allowed for

significantly more accurate identification of cardiovascular risk in COPD patients compared with clinical assessment alone. Among 1,218 extracted imaging parameters, 15 features with the highest diagnostic value were selected and, combined with age, body mass, and GOLD disease stage, formed a nomogram with stable and reproducible performance. The integrated model achieved AUC values of 0.731 in the training cohort, 0.727 in internal validation, and 0.725 in external validation, outperforming clinical models in all analyzed populations. Moreover, decision curve analysis confirmed greater clinical benefit of the image-clinical nomogram, suggesting that routine chest CT can be used not only for pulmonary evaluation but also for early stratification of cardiovascular risk in COPD patients.(Lin et al. 2024)[49]

Complementing these observations, further analyses have highlighted the importance of extrapulmonary information obtained from routine CT scans. In Lin et al. (2025), quantitative analysis of chest CT features allowed effective identification of coexisting cardiovascular disease in COPD patients. Nomograms based on pulmonary and mediastinal features significantly outperformed clinical models and visual assessment of coronary artery calcification scores (CACS) in diagnostic accuracy, achieving AUC values of 0.79 and 0.86 versus 0.71 for the clinical model and 0.65 for CACS. The highest performance was observed for the mediastinal feature-based model, highlighting the critical importance of extrapulmonary information in assessing cardiovascular risk in COPD. Decision curve analysis confirmed the higher clinical utility of imaging-based nomograms compared with traditional approaches, suggesting that routine chest CT may serve as a valuable tool for comprehensive evaluation of cardiovascular complications in this patient population.(Lin et al. 20240[50]

In summary, advanced quantitative analysis of whole-lung CT imaging represents an increasingly important tool in COPD diagnostics, enabling not only precise differentiation of patients from healthy individuals but also assessment of disease severity and identification of acute exacerbations. Radiomic models and nomograms integrating imaging features with clinical data demonstrate high diagnostic accuracy and clinical utility, surpassing traditional approaches based solely on clinical parameters. Moreover, routine chest CT, through analysis of both pulmonary and extrapulmonary structures, allows early stratification of cardiovascular risk in COPD patients, emphasizing its growing role as a comprehensive tool supporting individualized diagnosis and therapeutic management.

Differentiation of COPD and ACO

Differentiation between chronic obstructive pulmonary disease (COPD) and asthma-COPD overlap (ACO) represents a significant diagnostic challenge, requiring the use of both classical biomarkers and modern molecular tools. In the study by Keita Hirai et al. (2021), circulating microRNAs were shown to be useful in distinguishing the ACO phenotype from asthma and COPD. The authors identified five microRNAs (miR-148a-3p, miR-15b-5p, miR-223-3p, miR-23a-3p, and miR-26b-5p) whose expression was significantly decreased in patients with ACO, with miR-15b-5p demonstrating the highest diagnostic value for identifying this phenotype. Importantly, combining miR-15b-5p measurements with previously described type 2

inflammation biomarkers, namely periostin and YKL-40, significantly improved diagnostic accuracy compared with protein markers alone. These findings indicate that integrating microRNAs with classical serum biomarkers may enable more precise diagnosis and phenotyping of patients, which is particularly relevant in the context of asthma, COPD, and their overlapping form.(Keita Hirai et al. 2021)[51]

Similarly, the study by Chang et al. (2024) demonstrated a significant role of microRNA miR-125b-5p in the pathogenesis of ACO. In patients with ACO and in cellular models simultaneously stimulated with cigarette smoke and allergens, significant overexpression of miR-125b-5p was observed, accompanied by decreased expression of IL6R and TRIAP1 genes. These alterations were associated with increased late apoptosis of monocytes and elevated oxidative stress in bronchial epithelial cells, whereas silencing miR-125b-5p using siRNA reversed these deleterious effects. Additionally, a regulatory effect of miR-125b-5p on the STAT3 pathway in epithelial cells was demonstrated, suggesting that this microRNA may represent an important molecular element differentiating ACO and a potential target for future diagnostic and therapeutic interventions.(Chang et al. 2024)[52]

In the same study, Chang et al. (2024) also highlighted the critical role of the miR-23a-5p/RAGE/ROS signaling axis in the pathogenesis of cigarette smoke–induced COPD. In COPD patients, animal models, and bronchial epithelial cells exposed to cigarette smoke, significant overexpression of the receptor for advanced glycation end-products (RAGE) was observed, accompanied by decreased levels of miR-23a-5p. Upregulation of miR-23a-5p led to RAGE suppression, reduction of oxidative stress, inhibition of ERK pathway activation, and decreased pro-inflammatory cytokine production, resulting in attenuation of airway inflammation and improved lung function in COPD models. These results indicate that miR-23a-5p acts as an endogenous inhibitor of the RAGE-dependent axis and may represent a promising therapeutic target in COPD, particularly in tobacco smoke–induced disease.(Chang et al. 2024)[53]

Regarding classical biomarkers, Shirai et al. (2019) evaluated the utility of serum markers in differentiating ACO from isolated asthma and COPD. Periostin, a type 2 inflammation marker, was elevated in asthma and ACO patients but not in COPD, whereas YKL-40, characteristic for COPD, was increased in COPD and ACO but not in asthma patients. Notably, the proportion of patients with simultaneously high periostin and YKL-40 levels was significantly greater in the ACO group than in the other groups, highlighting the potential diagnostic value of combined measurement of both markers.(Shirai et al. 2019)[54]

The study by Wang et al. (2018) further confirmed the utility of plasma biomarkers in differentiating patients with asthma, COPD, and ACO. Patients with ACO exhibited lower YKL-40 levels compared with COPD and moderately elevated NGAL levels compared with asthma patients. Concurrently, this group showed an intermediate degree of airway obstruction and emphysema severity, positioned between the typical asthma and COPD profiles. Correlation analysis demonstrated that YKL-40 levels negatively correlated with pulmonary function parameters, whereas NGAL levels positively correlated with emphysema extent in imaging studies, indicating the usefulness of these biomarkers in distinguishing ACO phenotypes.(Wang et al. 2018)[55]

Conversely, the study by Bersimbaeva et al. (2021) emphasized the significance of circulating plasma microRNAs as potential biomarkers for asthma, COPD, and ACO. Distinct expression profiles of the analyzed microRNAs were observed across disease entities: hsa-miR-19b-3p was decreased in asthma and ACO but increased in COPD; hsa-miR-125b-5p showed reduced expression in COPD and increased levels in asthma and ACO; while hsa-miR-320c was decreased in asthma and elevated in COPD and ACO. ROC curve analysis confirmed high diagnostic value for these molecules, with the highest sensitivity and specificity observed for hsa-miR-19b-3p in asthma, hsa-miR-125b-5p in ACO, and hsa-miR-320c in COPD. These findings suggest that selected microRNAs may serve as promising, non-invasive biomarkers for differentiating chronic inflammatory airway diseases.(Bersimbaeva et al. 2021)[56]

Patients with asthma-COPD overlap (ACO) experience more frequent exacerbations and worse prognosis than those with asthma or COPD alone, making the identification of specific biomarkers for this phenotype clinically relevant. In a study comparing blood eosinophil counts, IgE levels, and plasma protein profiles in patients with asthma, COPD, ACO, and healthy controls (n=397, age 40–90 years), no differences in eosinophil counts were observed among groups. IgE levels were higher in ACO compared with COPD, and ten plasma proteins differentiated ACO from controls. In individuals over 60 years, CXCL9 effectively distinguished ACO from asthma (AUC 0.73), whereas in those under 60 years, MCP-3 differentiated ACO from COPD (AUC 0.84). CDCP1 levels and age >60 years were negatively associated with ACO. These findings suggest that CXCL9 and MCP-3 may serve as potential biomarkers differentiating ACO from asthma and COPD, and that the plasma inflammatory profile in ACO resembles that of COPD, pointing to novel diagnostic and potentially therapeutic targets.(Escamilla-Gil et al. 2025)[57]

Differentiation of COPD and ACO relies on integrating classical serum biomarkers, such as periostin, YKL-40, and NGAL, with modern molecular tools, including circulating microRNAs. Studies have shown that specific microRNAs (miR-15b-5p, miR-125b-5p, miR-23a-5p, hsa-miR-19b-3p, hsa-miR-320c) and combinations of protein and microRNA markers effectively distinguish the ACO phenotype from asthma and COPD, while identifying pathogenetic mechanisms related to oxidative stress, apoptosis, and airway inflammation. Combining molecular analyses with classical clinical indicators allows more precise patient phenotyping, improves diagnostic accuracy, and identifies potential therapeutic targets in the management of chronic obstructive airway diseases.

Discussion

Chronic obstructive pulmonary disease (COPD) remains a major global health challenge, characterized by progressive airflow limitation and systemic inflammation. While spirometry continues to be the classical diagnostic standard, emerging evidence highlights the increasing role of molecular, cellular, and imaging biomarkers in the assessment and management of COPD (Christenson, 2023; Xie et al., 2025)[10,11]. These novel diagnostic strategies allow not only early detection of disease and exacerbations but also identification of distinct phenotypes and endotypes, which are critical for individualized therapeutic decisions and prognosis (Zhang et al., 2023; Shi-Xia Liao et al., 2025)[13,14].

Peripheral blood represents a minimally invasive and readily accessible source of biomarkers. Markers such as neutrophil-to-lymphocyte ratio (NLR), eosinophil counts, and specific proteomic/metabolomic profiles have been shown to reflect systemic inflammation, disease severity, exacerbation risk, and therapeutic response (Fang et al., 2023; Li Fang et al., 2025; Papaporfyriou et al., 2022; Zhang et al., 2023)[12,13,16,17]. NLR, in particular, has been consistently associated with short-term mortality, major clinical events during hospitalization, and treatment monitoring in acute exacerbations of COPD (Zinellu et al., 2022; Yao et al., 2017; Vu-Hoai et al., 2024)[24,25,27]. Concurrently, eosinophil counts serve as reliable prognostic and theranostic biomarkers, predicting response to inhaled corticosteroids and biologics, as well as risk of recurrent exacerbations (Kang et al., 2021; Zhanga et al., 2020; Kiani et al., 2023; Pu et al., 2023; Citgez et al., 2021)[18,19,21,22,23].

Urinary biomarkers offer an additional non-invasive approach for COPD monitoring. Proteomic and metabolic analyses of urine reflect systemic and pulmonary pathophysiological changes, correlate with symptom burden and functional impairment, and enable early prediction of exacerbations (Fawzy et al., 2023; Kim et al., 2022; Yousuf et al., 2025; Shi et al., 2025)[30,31,32,33]. These findings suggest that daily or frequent urinary monitoring could facilitate early therapeutic intervention and improve disease management.

Respiratory microbiome analysis further enhances understanding of COPD heterogeneity. Dysbiosis, reduced bacterial diversity, and dominance of specific taxa such as Proteobacteria have been associated with more severe disease and higher exacerbation risk (Liu et al., 2021; Su et al., 2022; Bahetjan et al., 2025)[34,35,36]. Infections with *Pseudomonas aeruginosa* are particularly deleterious, contributing to enhanced neutrophil extracellular trap (NET) formation, oxidative stress, and worse clinical outcomes (Lin et al., 2025; Verceles et al., 2021)[38,40]. Integrating microbiome with proteomic and metabolomic data allows identification of potential diagnostic and prognostic biomarkers, as well as novel therapeutic targets.

Genetic and transcriptomic analyses provide insights into molecular mechanisms underlying COPD. Multi-omics studies reveal epigenetic alterations, differential gene expression, and immune cell dysregulation, identifying potential diagnostic and therapeutic targets such as PRPF19, PPIB, EXPH5, STAU1, and SLC27A3 (Zhao et al., 2025; Hui Yu et al., 2021; Yang et al., 2023; Zhang et al., 2022)[42,43,44,45]. Integrating these molecular signatures with clinical and inflammatory data enables more precise differentiation of disease stages and assessment of individual patient prognosis.

Advanced imaging techniques, particularly quantitative computed tomography (CT) and radiomics, allow detailed assessment of structural lung changes, disease severity, and acute exacerbations (Zhou et al., 2024; Zhou et al., 2025; Lin et al., 2024)[46,48,49]. CT-based models also provide valuable information on extrapulmonary complications, such as cardiovascular disease, further supporting comprehensive patient stratification and personalized management (Lin et al., 2025)[50].

Differentiation between COPD and asthma-COPD overlap (ACO) remains challenging. Combining classical biomarkers, such as periostin, YKL-40, and NGAL, with circulating

microRNAs (e.g., miR-15b-5p, miR-125b-5p, miR-23a-5p) improves diagnostic precision, enables identification of overlapping phenotypes, and informs targeted therapeutic approaches (Keita Hirai et al., 2021; Chang et al., 2024; Shirai et al., 2019; Escamilla-Gil et al., 2025)[51,52,54,57].

Overall, the integration of clinical, imaging, molecular, and microbiome data represents a promising strategy to address the heterogeneity of COPD. Multi-omics approaches, dynamic phenotyping, and evaluation of "treatable traits" provide opportunities for precision medicine, allowing more accurate diagnosis, individualized therapy, and improved prognostic assessment (Christenson, 2023; Xie et al., 2025)[10,11]. Despite these advances, challenges remain regarding standardization of biomarker use, reproducibility across cohorts, and translation into routine clinical practice, underscoring the need for further large-scale, prospective studies.

Conclusions

Chronic obstructive pulmonary disease (COPD) is a heterogeneous condition characterized by complex pathophysiological, molecular, and clinical variability. While spirometry remains the cornerstone of diagnosis, emerging diagnostic strategies—including blood, urine, and sputum biomarkers, advanced imaging, genetic and transcriptomic analyses, and microbiome profiling—provide complementary insights that enhance early detection, prognostic stratification, and personalized management.

Blood-based markers such as neutrophil-to-lymphocyte ratio (NLR) and eosinophil counts, alongside proteomic and metabolomic profiles, enable identification of disease severity, exacerbation risk, and therapeutic responsiveness. Urinary biomarkers offer a non-invasive means to monitor metabolic and inflammatory changes, predict exacerbations, and assess environmental risk factors. Respiratory microbiome analysis highlights the role of dysbiosis and specific pathogens, such as *Pseudomonas aeruginosa*, in disease progression and acute exacerbations. Genetic and transcriptomic studies elucidate key molecular mechanisms and potential targets for precision therapy, while quantitative CT and radiomics facilitate structural and functional phenotyping and assessment of extrapulmonary complications.

Integration of these multidimensional data supports the identification of clinically and molecularly defined phenotypes and endotypes, allowing tailored interventions based on "treatable traits." In particular, combining classical biomarkers with novel molecular tools improves differentiation of COPD from asthma-COPD overlap (ACO) and refines prognostic evaluation.

In summary, the future of COPD diagnostics and management lies in a personalized, multi-omics approach that combines traditional functional assessment with molecular, cellular, and imaging biomarkers. Such integration promises earlier detection, more accurate prognostic stratification, and optimized, individualized therapy, ultimately improving patient outcomes and reducing disease burden.

Author's contribution

Conceptualization: Konrad Gawin, Wiktoria Zawiślak, Maria Dąbrowska

Methodology: Daria Mrozik-Gałecka, Jolanta Cholewińska-Rychlica, Anita Ignasiak

Software: Kacper Rychlica, Michał Cisowski

Check: Konrad Gawin, Jolanta Cholewińska-Rychlica

Formal analysis: Michał Cisowski, Paulina Madura, Daria Mrozik-Gałecka

Investigation: Wiktoria Zawiślak, Maria Dąbrowska, Anita Ignasiak, Daria Mrozik-Gałecka

Resources: Jolanta Cholewińska-Rychlica, Kacper Rychlica, Wiktoria Zawiślak

Data curation: Paulina Madura, Anita Ignasiak

Writing- rough preparation: Jolanta Cholewińska-Rychlica, Kacper Rychlica, Daria Mrozik-Gałecka, Paulina Madura

Writing- review and editing: Konrad Gawin, Maria Dąbrowska, Wiktoria Zawiślak, Michał Cisowski

Visualization: Kacper Rychlica, Maria Dąbrowska, Anita Ignasiak.

Supervison: Anita Ignasiak, Kacper Rychlica

Project administration Konrad Gawin, Wiktoria Zawiślak, Maria Dąbrowska

All authors have read and agreed with published version of the manuscript.

Financing statement:

This research received no external funding.

Institutional Review Board Statement:

Not applicable.

Informed Consent Statement:

Not applicable.

Data Availability Statement:

Not applicable.

Conflict of interest:

The authors deny any conflict of interest.

Declaration of the use of generative AI and AI-assisted technologies the writing process.

While preparing this manuscript, the authors used the ChatGPT tool to enhance language quality and readability. After using the tool, the authors thoroughly reviewed and edited the text as necessary and take full responsibility for the scientific content of the publication.

References

1. GBD Chronic Respiratory Disease Collaborators. Prevalence and attributable health burden of chronic respiratory diseases, 1990-2017: a systematic analysis for the Global Burden of Disease Study 2017. Lancet Respir Med. 2020 Jun;8(6):585-596. doi: 10.1016/S2213-2600(20)30105-3. PMID: 32526187; PMCID: PMC7284317. https://doi.org/10.1016/s2213-2600(20)30105-3

2. Adeloye D, Song P, Zhu Y, Campbell H, Sheikh A, Rudan I; NIHR RESPIRE Global Respiratory Health Unit. Global, regional, and national prevalence of, and risk factors for, chronic obstructive pulmonary disease (COPD) in 2019: a systematic review and modelling analysis. Lancet Respir Med. 2022 May;10(5):447-458. doi: 10.1016/S2213-2600(21)00511-7. Epub 2022 Mar 10. PMID: 35279265; PMCID: PMC9050565. https://doi.org/10.1016/s2213-2600(21)00511-7

3. Adeloye D, Chua S, Lee C, Basquill C, Papana A, Theodoratou E, Nair H, Gasevic D, Sridhar D, Campbell H, Chan KY, Sheikh A, Rudan I; Global Health Epidemiology Reference Group (GHERG). Global and regional estimates of COPD prevalence: Systematic review and meta-analysis. J Glob Health. 2015 Dec;5(2):020415. doi: 10.7189/jogh.05.020415. PMID: 26755942; PMCID: PMC4693508. https://doi.org/10.7189/jogh.05.020415

4. Celli B, Fabbri L, Criner G, Martinez FJ, Mannino D, Vogelmeier C, Montes de Oca M, Papi A, Sin DD, Han MK, Agusti A. Definition and Nomenclature of Chronic Obstructive Pulmonary Disease: Time for Its Revision. Am J Respir Crit Care Med. 2022 Dec 1;206(11):1317-1325. doi: 10.1164/rccm.202204-0671PP. PMID: 35914087; PMCID: PMC9746870. https://doi.org/10.1164/rccm.202204-0671pp

5. World Health Organization. (2023, November 2). Chronic obstructive pulmonary disease (COPD). https://www.who.int/news-room/fact-sheets/detail/chronic-obstructive-pulmonary-disease-(copd)

6. Szczeklik, A., Gajewski, P. (Red.). (2024). Interna Szczeklika 2024. Medycyna Praktyczna.

7. Barnes PJ. Against the Dutch hypothesis: asthma and chronic obstructive pulmonary disease

are distinct diseases. Am J Respir Crit Care Med. 2006 Aug 1;174(3):240-3; discussion 243-4. doi: 10.1164/rccm.2604008. PMID: 16864717. https://doi.org/10.1164/rccm.2604008

8. Global Initiative for Chronic Obstructive Lung Disease (GOLD). Global strategy for the diagnosis, management, and prevention of chronic obstructive pulmonary disease. Updated 2025. http://www.goldcopd.com

9. Schivo, M., Albertson, T. E., Haczku, A., Kenyon, N. J., Zeki, A. A., Kuhn, B. T., Louie, S., & Avdalovic, M. V. (2017). Paradigms in chronic obstructive pulmonary disease: phenotypes, immunobiology, and therapy with a focus on vascular disease. Journal of investigative medicine : the official publication of the American Federation for Clinical Research, 65(6), 953–963. https://doi.org/10.1136/jim-2016-000358

10. Christenson S. A. (2023). COPD Phenotyping. Respiratory care, 68(7), 871–880. https://doi.org/10.4187/respcare.11035

11. Xie, C., Wang, K., Yang, K., Zhong, Y., Gul, A., Luo, W., Yalikun, M., He, J., Chen, W., Xu, W., & Dong, J. (2025). Toward precision medicine in COPD: phenotypes, endotypes, biomarkers, and treatable traits. Respiratory research, 26(1), 274. https://doi.org/10.1186/s12931-025-03356-w

12. Fang H, Liu Y, Yang Q, Han S, Zhang H. Prognostic Biomarkers Based on Proteomic Technology in COPD: A Recent Review. Int J Chron Obstruct Pulmon Dis. 2023 Jun 30;18:1353-1365. doi: 10.2147/COPD.S410387. PMID: 37408604; PMCID: PMC10319291. https://doi.org/10.2147/copd.s410387

13. Zhang Z, Wang J, Li Y, Liu F, Chen L, He S, Lin F, Wei X, Fang Y, Li Q, Zhou J, Lu W. Proteomics and metabolomics profiling reveal panels of circulating diagnostic biomarkers and molecular subtypes in stable COPD. Respir Res. 2023 Mar 11;24(1):73. doi: 10.1186/s12931-023-02349-x. PMID: 36899372; PMCID: PMC10007826. https://doi.org/10.1186/s12931-023-02349-x

14. Liao SX, Wang YW, Sun PP, Xu Y, Wang TH. Prospects of neutrophilic implications against pathobiology of chronic obstructive pulmonary disease: Pharmacological insights and technological advances. Int Immunopharmacol. 2025 Jan 10;144:113634. doi: 10.1016/j.intimp.2024.113634. Epub 2024 Nov 21. PMID: 39577220. https://doi.org/10.1016/j.intimp.2024.113634

15. Huang Y, Niu Y, Wang X, Li X, He Y, Liu X. Identification of novel biomarkers related to neutrophilic inflammation in COPD. Front Immunol. 2024 May 30;15:1410158. doi: 10.3389/fimmu.2024.1410158. PMID: 38873611; PMCID: PMC11169582. https://doi.org/10.3389/fimmu.2024.1410158

16. Fang L, Zhu J, Fu D. Predictive value of neutrophil-lymphocyte ratio for all-cause mortality in patients with chronic obstructive pulmonary disease: a systematic review and meta-analysis. BMC Pulm Med. 2025 Apr 29;25(1):206. doi: 10.1186/s12890-025-03677-y. PMID: 40301774;

PMCID: PMC12039089. https://doi.org/10.1186/s12890-025-03677-y

17. Papaporfyriou A, Bakakos P, Hillas G, Papaioannou AI, Loukides S. Blood eosinophils in COPD: friend or foe? Expert Rev Respir Med. 2022 Jan;16(1):35-41. doi: 10.1080/17476348.2021.2011219. Epub 2021 Dec 3. PMID: 34821191.

18. Kang, H. S., Kim, S. K., Kim, Y. H., Kim, J. W., Lee, S. H., Yoon, H. K., & Rhee, C. K. (2021). The association between eosinophilic exacerbation and eosinophilic levels in stable COPD. BMC pulmonary medicine, 21(1), 74. https://doi.org/10.1186/s12890-021-01443-4

19. Zhang, Y., Liang, L. R., Zhang, S., Lu, Y., Chen, Y. Y., Shi, H. Z., & Lin, Y. X. (2020). Blood Eosinophilia and Its Stability in Hospitalized COPD Exacerbations are Associated with Lower Risk of All-Cause Mortality. International journal of chronic obstructive pulmonary disease, 15, 1123–1134. https://doi.org/10.2147/COPD.S245056

20. Liu, H., Xie, Y., Huang, Y., Luo, K., Gu, Y., Zhang, H., Xu, Y., & Chen, X. (2024). The association between blood eosinophils and clinical outcome of acute exacerbations of chronic obstructive pulmonary disease: A systematic review and meta-analysis. Respiratory medicine, 222, 107501. https://doi.org/10.1016/j.rmed.2023.107501

21. Kiani, A., Rahimi, F., Afaghi, S., Paat, M., Varharam, M., Dizaji, M. K., Dastoorpoor, M., & Abedini, A. (2023). Association of Upon-Diagnosis Blood Eosinophilic Count with Frequency and Severity of Annual Exacerbation in Chronic Obstructive Pulmonary Disease: A Prospective Longitudinal Analysis. Canadian respiratory journal, 2023, 8678702. https://doi.org/10.1155/2023/8678702

22. Pu, J., Yi, Q., Luo, Y., Wei, H., Ge, H., Liu, H., Li, X., Zhang, J., Pan, P., Zhou, H., Zhou, C., Yi, M., Cheng, L., Liu, L., Zhang, J., Peng, L., Aili, A., Liu, Y., Zhou, H., & MAGNET AECOPD Registry Investigators (2023). Blood Eosinophils and Clinical Outcomes in Inpatients with Acute Exacerbation of Chronic Obstructive Pulmonary Disease: A Prospective Cohort Study. International journal of chronic obstructive pulmonary disease, 18, 169–179. https://doi.org/10.2147/COPD.S396311

23. Citgez, E., van der Palen, J., van der Valk, P., Kerstjens, H. A. M., & Brusse-Keizer, M. (2021). Stability in eosinophil categorisation during subsequent severe exacerbations of COPD. BMJ open respiratory research, 8(1), e000960. https://doi.org/10.1136/bmjresp-2021-000960

24. Zinellu, A., Zinellu, E., Mangoni, A. A., Pau, M. C., Carru, C., Pirina, P., & Fois, A. G. (2022). Clinical significance of the neutrophil-to-lymphocyte ratio and platelet-to-lymphocyte ratio in acute exacerbations of COPD: present and future. European respiratory review : an official journal of the European Respiratory Society, 31(166), 220095. https://doi.org/10.1183/16000617.0095-2022

25. Yao, C., Liu, X., & Tang, Z. (2017). Prognostic role of neutrophil-lymphocyte ratio and platelet-lymphocyte ratio for hospital mortality in patients with AECOPD. International journal of chronic obstructive pulmonary disease, 12, 2285–2290.

https://doi.org/10.2147/COPD.S141760

26. Jiang, M., Yang, Y., & Wang, H. (2024). Stability of Neutrophil to Lymphocyte Ratio in Acute Exacerbation of Chronic Obstructive Pulmonary Disease and Its Relationship with Clinical Outcomes: A Retrospective Cohort Study. International journal of chronic obstructive pulmonary disease, 19, 2431–2441. https://doi.org/10.2147/COPD.S487063

27. Vu-Hoai, N., Ly-Phuc, D., Duong-Minh, N., Tran-Ngoc, N., & Nguyen-Dang, K. (2024). Predictive value of neutrophil-to-lymphocyte ratio for adverse outcomes in hospitalized patients with acute exacerbation of chronic obstructive pulmonary disease: A retrospective study. Medicine, 103(38), e39797. https://doi.org/10.1097/MD.0000000000039797

28. Feng, X., Xiao, H., Duan, Y., Li, Q., & Ou, X. (2023). Prognostic Value of Neutrophil to Lymphocyte Ratio for Predicting 90-Day Poor Outcomes in Hospitalized Patients with Acute Exacerbation of Chronic Obstructive Pulmonary Disease. International journal of chronic obstructive pulmonary disease, 18, 1219–1230. https://doi.org/10.2147/COPD.S399671

29. Fang, H., Liu, Y., Yang, Q., Han, S., & Zhang, H. (2023). Prognostic Biomarkers Based on Proteomic Technology in COPD: A Recent Review. International journal of chronic obstructive pulmonary disease, 18, 1353–1365. https://doi.org/10.2147/COPD.S410387

30. Fawzy, A., Putcha, N., Raju, S., Woo, H., Lin, C. T., Brown, R. H., Williams, M. S., Faraday, N., McCormack, M. C., & Hansel, N. (2023). Urine and Plasma Markers of Platelet Activation and Respiratory Symptoms in COPD. Chronic obstructive pulmonary diseases (Miami, Fla.), 10(1), 22–32. https://doi.org/10.15326/jcopdf.2022.0326

31. Kim, H. Y., Lee, H. S., Kim, I. H., Kim, Y., Ji, M., Oh, S., Kim, D. Y., Lee, W., Kim, S. H., & Paik, M. J. (2022). Comprehensive Targeted Metabolomic Study in the Lung, Plasma, and Urine of PPE/LPS-Induced COPD Mice Model. International journal of molecular sciences, 23(5), 2748. https://doi.org/10.3390/ijms23052748

32. Yousuf, A. J., Parekh, G., Farrow, M., Ball, G., Graziadio, S., Wilson, K., Lendrem, C., Carr, L., Watson, L., Parker, S., Finch, J., Glover, S., Mistry, V., Porter, K., Duvoix, A., O'Brien, L., Rees, S., Lewis, K. E., Davis, P., & Brightling, C. E. (2025). Artificial neural network risk prediction of COPD exacerbations using urine biomarkers. ERJ open research, 11(3), 00797-2024. https://doi.org/10.1183/23120541.00797-2024

33. Shi, Y., Pu, S., Huang, N., & Wang, Y. (2025). Association Between Urinary Glyphosate Concentrations and Chronic Obstructive Pulmonary Disease in USA Participants: Evidence from NHANES 2013-2018. International journal of chronic obstructive pulmonary disease, 20, 883–894. https://doi.org/10.2147/COPD.S500429

34. Liu, J., Ran, Z., Wang, F., Xin, C., Xiong, B., & Song, Z. (2021). Role of pulmonary microorganisms in the development of chronic obstructive pulmonary disease. Critical reviews in microbiology, 47(1), 1–12. https://doi.org/10.1080/1040841X.2020.1830748

35. Su, L., Qiao, Y., Luo, J., Huang, R., Li, Z., Zhang, H., Zhao, H., Wang, J., & Xiao, Y. (2022). Characteristics of the sputum microbiome in COPD exacerbations and correlations between clinical indices. Journal of translational medicine, 20(1), 76. https://doi.org/10.1186/s12967-022-03278-x

36. Bahetjan, K., Yu-Xia, Lin, S., Aili, N., Yang, H., & Du, S. (2025). Analysis of the bronchoalveolar lavage fluid microbial flora in COPD patients at different lung function during acute exacerbation. Scientific reports, 15(1), 13179. https://doi.org/10.1038/s41598-025-96746-5

37. Yang, C. Y., Li, S. W., Chin, C. Y., Hsu, C. W., Lee, C. C., Yeh, Y. M., & Wu, K. A. (2021). Association of exacerbation phenotype with the sputum microbiome in chronic obstructive pulmonary disease patients during the clinically stable state. Journal of translational medicine, 19(1), 121. https://doi.org/10.1186/s12967-021-02788-4

38. Lin, Z., Xue, M., Lu, M., Liu, S., Jiang, Y., Yang, Q., Cui, H., Huang, X., Zheng, Z., & Sun, B. (2025). Multi-omics driven biomarker discovery and pathological insights into Pseudomonas aeruginosa pneumonia. BMC infectious diseases, 25(1), 745. https://doi.org/10.1186/s12879-025-11119-7

39. Lin, Z., Liu, S., Zhang, K., Feng, T., Luo, Y., Liu, Y., Sun, B., & Zhou, L. (2025). Molecular mechanisms and therapeutic targets of acute exacerbations of chronic obstructive pulmonary disease with Pseudomonas aeruginosa infection. Respiratory research, 26(1), 115. https://doi.org/10.1186/s12931-025-03185-x

40. Verceles, A. C., Bhat, P., Nagaria, Z., Martin, D., Patel, H., Ntem-Mensah, A., Hyun, S. W., Hahn, A., Jeudy, J., Cross, A. S., Lillehoj, E. P., & Goldblum, S. E. (2021). MUC1 ectodomain is a flagellin-targeting decoy receptor and biomarker operative during Pseudomonas aeruginosa lung infection. Scientific reports, 11(1), 22725. https://doi.org/10.1038/s41598-021-02242-x

41. Zhang Y, Sheng Y, Gao Y, Lin Y, Cheng B, Li H, Zhang L, Xu H. Exploration of the Pathogenesis of Chronic Obstructive Pulmonary Disease Caused by Smoking-Based on Bioinformatics Analysis and In Vitro Experimental Evidence. Toxics. 2023 Dec 7;11(12):995. doi: 10.3390/toxics11120995. PMID: 38133396; PMCID: PMC10747869. https://doi.org/10.3390/toxics11120995

42. Zhao J, Ge X, Li H, Jing G, Ma W, Fan Y, Chen J, Zhao Z, Hou J. Hub Genes PRPF19 and PPIB: Molecular Pathways and Potential Biomarkers in COPD. Int J Chron Obstruct Pulmon Dis. 2025 Jun 11;20:1865-1880. doi: 10.2147/COPD.S511696. PMID: 40524719; PMCID: PMC12168939.

43. Yu H, Guo W, Liu Y, Wang Y. Immune Characteristics Analysis and Transcriptional Regulation Prediction Based on Gene Signatures of Chronic Obstructive Pulmonary Disease. Int J Chron Obstruct Pulmon Dis. 2021 Nov 5;16:3027-3039. doi: 10.2147/COPD.S325328. PMID: 34764646; PMCID: PMC8577508. https://doi.org/10.2147/copd.s325328

44. Yang Y, Cao Y, Han X, Ma X, Li R, Wang R, Xiao L, Xie L. Revealing EXPH5 as a potential diagnostic gene biomarker of the late stage of COPD based on machine learning analysis. Comput Biol Med. 2023 Mar;154:106621. doi: 10.1016/j.compbiomed.2023.106621. Epub 2023 Jan 31. PMID: 36746116. https://doi.org/10.1016/j.compbiomed.2023.106621

45. Zhang Y, Xia R, Lv M, Li Z, Jin L, Chen X, Han Y, Shi C, Jiang Y, Jin S. Machine-Learning Algorithm-Based Prediction of Diagnostic Gene Biomarkers Related to Immune Infiltration in Patients With Chronic Obstructive Pulmonary Disease. Front Immunol. 2022 Mar 8;13:740513. doi: 10.3389/fimmu.2022.740513. PMID: 35350787; PMCID: PMC8957805. https://doi.org/10.3389/fimmu.2022.740513

46. Zhou, T. H., Zhou, X. X., Ni, J., Ma, Y. Q., Xu, F. Y., Fan, B., Guan, Y., Jiang, X. A., Lin, X. Q., Li, J., Xia, Y., Wang, X., Wang, Y., Huang, W. J., Tu, W. T., Dong, P., Li, Z. B., Liu, S. Y., & Fan, L. (2024). CT whole lung radiomic nomogram: a potential biomarker for lung function evaluation and identification of COPD. Military Medical Research, 11(1), 14. https://doi.org/10.1186/s40779-024-00516-9

47. Zhou, T., Zhou, X., Ni, J., Guan, Y., Jiang, X., Lin, X., Li, J., Xia, Y., Wang, X., Wang, Y., Huang, W., Tu, W., Dong, P., Li, Z., Liu, S., & Fan, L. (2024). A CT-Based Lung Radiomics Nomogram for Classifying the Severity of Chronic Obstructive Pulmonary Disease. International journal of chronic obstructive pulmonary disease, 19, 2705–2717. https://doi.org/10.2147/COPD.S483007

48. Zhou, X., Ma, Y., Zhou, T., Xie, X., Li, Y., Guan, Y., Wang, Y., Li, J., Zhang, H., Liu, S., & Fan, L. (2025). A computed tomography-based lung radiomics nomogram to identify acute exacerbation of chronic obstructive pulmonary disease: a multi-institutional validation study. Journal of thoracic disease, 17(10), 7762–7777. https://doi.org/10.21037/jtd-2025-972

49. Lin, X., Zhou, T., Ni, J., Li, J., Guan, Y., Jiang, X., Zhou, X., Xia, Y., Xu, F., Hu, H., Dong, Q., Liu, S., & Fan, L. (2024). CT-based whole lung radiomics nomogram: a tool for identifying the risk of cardiovascular disease in patients with chronic obstructive pulmonary disease. European radiology, 34(8), 4852–4863. https://doi.org/10.1007/s00330-023-10502-9

50. Lin, X., Zhou, T., Ni, J., Zhou, X., Guan, Y., Jiang, X., Xia, Y., Xu, F., Hu, H., Li, J., Zhang, J., Liu, S., Vliegenthart, R., & Fan, L. (2025). CT-Based radiomics nomogram of lung and mediastinal features to identify cardiovascular disease in chronic obstructive pulmonary disease: a multicenter study. BMC pulmonary medicine, 25(1), 121. https://doi.org/10.1186/s12890-025-03568-2

51. Hirai K, Shirai T, Shimoshikiryo T, Ueda M, Gon Y, Maruoka S, Itoh K. Circulating microRNA-15b-5p as a biomarker for asthma-COPD overlap. Allergy. 2021 Mar;76(3):766-774. doi: 10.1111/all.14520. Epub 2020 Aug 20. PMID: 32713026. https://doi.org/10.1111/all.14520

52. Chang YP, Tsai YH, Chen YM, Huang KT, Lee CP, Hsu PY, Chen HC, Lin MC, Chen YC. Upregulated microRNA-125b-5p in patients with asthma-COPD overlap mediates oxidative

stress and late apoptosis via targeting IL6R/TRIAP1 signaling. Respir Res. 2024 Feb 1;25(1):64. doi: 10.1186/s12931-024-02703-7. PMID: 38302925; PMCID: PMC10835813. https://doi.org/10.1186/s12931-024-02703-7

53. Chang C, Huang K, Xu X, Duan R, Yu T, Chu X, Chen C, Li B, Yang T. MiR-23a-5p alleviates chronic obstructive pulmonary disease through targeted regulation of RAGE-ROS pathway. Respir Res. 2024 Feb 20;25(1):93. doi: 10.1186/s12931-024-02736-y. PMID: 38378600; PMCID: PMC10880325. https://doi.org/10.1186/s12931-024-02736-y

54. Shirai, T., Hirai, K., Gon, Y., Maruoka, S., Mizumura, K., Hikichi, M., Holweg, C., Itoh, K., Inoue, H., & Hashimoto, S. (2019). Combined Assessment of Serum Periostin and YKL-40 May Identify Asthma-COPD Overlap. The journal of allergy and clinical immunology. In practice, 7(1), 134–145.e1. https://doi.org/10.1016/j.jaip.2018.06.015

55. Wang, J., Lv, H., Luo, Z., Mou, S., Liu, J., Liu, C., Deng, S., Jiang, Y., Lin, J., Wu, C., Liu, X., He, J., & Jiang, D. (2018). Plasma YKL-40 and NGAL are useful in distinguishing ACO from asthma and COPD. Respiratory research, 19(1), 47. https://doi.org/10.1186/s12931-018-0755-6

56. Bersimbaev, R., Aripova, A., Bulgakova, O., Kussainova, A., Akparova, A., & Izzotti, A. (2021). The Plasma Levels of hsa-miR-19b-3p, hsa-miR-125b-5p, and hsamiR- 320c in Patients with Asthma, COPD and Asthma-COPD Overlap Syndrome (ACOS). MicroRNA (Shariqah, United Arab Emirates), 10(2), 130–138. https://doi.org/10.2174/2211536610666210609142859

57. Escamilla-Gil, J. M., Torres-Duque, C. A., Llinás-Caballero, K., Proaños-Jurado, N. J., De Vivero, M. M., Ramirez, J. C., Regino, R., Florez de Arco, L. T., Dennis, R., González-García, M., Caraballo, L., & Acevedo, N. (2025). Plasma Levels of CXCL9 and MCP-3 are Increased in Asthma-COPD Overlap (ACO) Patients. International journal of chronic obstructive pulmonary disease, 20, 1161–1174. https://doi.org/10.2147/COPD.S506517