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Acute COPD exacerbation despite triple inhaled therapy: a molecular insight – TripleEx study

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Abstract

Background Chronic obstructive pulmonary disease (COPD) is a progressive respiratory disorder characterized by persistent airflow limitation and acute exacerbations (AECOPD), which accelerate disease progression. Although triple inhaled therapy is recommended for patients with severe COPD and frequent AECOPD, some patients continue to experience exacerbations. The mechanisms behind this remain unclear. Exhaled breath analysis has the potential to unravel molecular changes during AECOPD, thereby adding to the understanding of molecular drivers for AECOPD. This study aimed to investigate metabolic changes in exhaled breath during AECOPD compared to stable state.

Methods In COPD patients treated with triple inhaled therapy we conducted real time breath analysis during AECOPD and subsequent stable state. Molecular breath patterns were compared between AECOPD and stable state by pathway enrichment analysis. Minimum description length model was used to build a feature based prediction model differentiating AECOPD from stable state.

Results 28 patients (61% male) with a mean (SD) age of 65 (10.2) years with severe AECOPD were included. Metabolic alterations were predominantly detected in aminosugar, linoleate, and butanoate pathways. AECOPD could be discriminated from stable state with high power (AUC = 0.84), and balanced good sensitivity and specificity (86% each).

Conclusion Metabolic analysis of AECOPD revealed disturbances in aminosugar metabolism as a potential driver mechanism and thus may be a therapeutic target for patients with exacerbations despite triple inhaled therapy. Moreover, real-time breath analysis could enable rapid detection of AECOPD, improving diagnostic accuracy and treatment efficiency.

Trial registration ClinicalTrials.gov (NCT04638920), registered on 20.11.2020.

Keywords COPD, Exacerbation, Breath research, Biomarkers, Triple inhaled therapy, SESI-HRMS

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Background

Chronic obstructive pulmonary disease (COPD) is a prevalent and progressive respiratory disorder characterised by persistent airflow limitation and chronic inflammatory response in the airways and lungs. The management of COPD is challenging due to its clinical heterogeneity and high morbidity and mortality [1]. Acute exacerbations of COPD (AECOPD), defined as episodes of acute worsening of respiratory symptoms beyond daily variations [2], significantly contribute to disease progression [3], reduced quality of life [4], and increased healthcare utilisation [5]. Triple inhaled therapy, which includes a combination of an inhaled corticosteroid (ICS), a long-acting beta-agonist (LABA), and a long-acting muscarinic antagonist (LAMA), is widely recommended for patients with severe disease and frequent exacerbations. The rationale behind this combination is to target different pathophysiological mechanisms: ICS reduces inflammation, LABA enhances bronchodilation by stimulating beta-2 adrenergic receptors, and LAMA blocks muscarinic receptors to reduce bronchoconstriction. Despite the effectiveness of this therapy in reducing the general frequency of exacerbations and improving lung function [6], a subset of patients continues to experience exacerbations while on triple inhaled therapy [7]. This incomplete control of exacerbations in COPD patients on triple inhaled therapy remains poorly understood, posing a significant challenge to clinical management and pharmacological development. It has been hypothesised that various factors, including bacterial and viral infections, different exacerbation phenotypes, underlying comorbidities, and systemic inflammation contribute to the incomplete control of exacerbations. Exploring these factors is critical for improving outcomes in COPD management and optimising treatment strategies to prevent exacerbations in patients receiving triple inhaled therapy.

Exhaled breath analysis emerges as a promising tool for decoding underlying pathomechanism, providing a non-invasive, real-time reflection of metabolic and biochemical processes in the body [8]. A recent study found that COPD patients with frequent exacerbations exhibit distinct molecular breath patterns compared to those without frequent exacerbations, primarily involving pathways related to inflammation and oxidative stress [9]. Furthermore, investigation of molecular breath patterns comparing AECOPD episodes to subsequent stable states in a COPD cohort with heterogeneous long-term inhaled therapy revealed alterations predominantly in molecular pathways related to acute inflammation [10]. However, which metabolic pathways are changed during AECOPD compared to stable state in patients on triple inhaled therapy and their potential for novel pharmacological treatment strategies have remained unclear. Therefore, our objective was to identify altered metabolic pathways during AECOPD in a longitudinal setting and evaluate their potential for additional pharmacological treatment strategies utilising real-time mass spectrometry. To achieve this, we investigated patients with COPD experiencing AECOPD despite triple inhaled therapy and analyzed their exhaled breath during AECOPD and a subsequent stable state.

Study design and methods

Study design and subjects

This prospective observational study was conducted at the University Hospital Zurich between November 2020 and April 2024 and included hospitalised patients with COPD on triple inhaled therapy experiencing AECOPD. Inclusion criteria were a minimum age of 18 years, a pre-existing clinical diagnosis of COPD, and an established triple inhaled therapy with ICS + LAMA + LABA. Patients who could not give informed consent or follow the protocol due to physical or intellectual limitations were excluded. Additionally, those receiving intensive care and pregnant individuals were excluded. Patients underwent two study visits. An initial visit (V1) during AECOPD (within 48 h of hospital admission), and a second visit (V2) after a recovery period of at least 8 weeks when patients were expected to have returned to a stable phase of their COPD. During both visits, comprehensive clinical data including markers of inflammation, such as high-sensitive C-reactive protein (hs-CRP), blood eosinophil levels, fractional exhaled nitric oxide (FeNO), microbiology (sputum culture or urine antigen for streptococcus pneumonia and legionella pneumophila), spirometry (forced expiratory volume in the first second (FEV1% predicted) and forced vital capacity (FVC % predicted)), venous blood oxygen saturation level, symptoms (COPD Assessment Test (CAT) and modified Medical Research Council Dyspnea Scale (mMRC)) were collected. Additionally, patients were asked to provide information regarding their medical history, including exacerbation history, current medications and adherence to triple inhaled therapy, smoking history as well as recent consumption of food, beverages, caffeine, cigarettes, alcohol, and cosmetics use. The study was approved by the cantonal ethics committee of Zurich (BASEC-Nr. 2020 – 01954) and adhered to the principles of the Declaration of Helsinki and Good Clinical Practice. The study was registered at ClinicalTrials.gov (NCT04638920) and all participants provided written informed consent.

Breath analysis

On-line breath analysis was performed using a Secondary Electrospray Ionization source (Fossil Ion Technology, Spain) coupled with Q Exactive Plus high-resolution mass spectrometry (Thermo Fisher Scientific, Germany)

(SESI-HRMS). Room temperature was kept constant throughout the procedure. The protocol for breath analysis has been previously described [11, 12]. Briefly, participants exhaled through a sterile filter connected to a heated tube (50 cm long and 4 mm in diameter, made of stainless steel and coated with Silconert) that was Linked to the curtain AUX gas port of an Orbitrap spectrometer. To prevent water condensation and compound loss on the tube walls, the tube was heated to 130 °C using insulated heating tape. A digital capnograph continuously monitored the volume in the sampling Line during exhalation. Breath samples were recorded in real time, with patients instructed to exhale without force six times in both positive and negative ionisation modes, thus a total of 12 exhalations resulted.

Spectra analysis

The initial dataset included 112 mass spectrometer and capnograph files (56 from patients during AECOPD and 56 from the stable COPD state, with 28 in positive and 28 negative ionization mode. All files were preprocessed using a custom pipeline developed by Deep Breath Intelligence (DBI, Switzerland), which included peak detection, alignment, and noise filtering. Mass spectral data were extracted directly from RAW files using proprietary software based on Thermo Fisher Scientific's RawFileReader. The resulting data matrix contained 3298 features, consisting of 2038 mass-to-charge ratios (m/z) in positive mode and 1260 in negative mode.

Statistics

To assess changes in breath feature intensities between V1 and V2, fold-change values were calculated by log-transforming the ratio of feature signal intensities in V1 to V2 (log2FC). Paired t-tests were used to evaluate differences in feature intensities between the two visits. P-values were adjusted for multiple comparison by computing their corresponding q-values [13].

Feature selection was performed using a cost-sensitive variant of the Minimum Description Length of ReliefF [14], where costs were introduced through sampling (CORElearn v1.57.3.1) and repeated 100 times with different training and testing datasets to avoid overfitting. A Naive Bayes machine learning algorithm (tidymodels v1.2.0) was then applied with the highest-ranking features to create prediction models based on breath features. Hyperparameters were tuned to achieve the best prediction performance. 100-fold cross-validations were computed and the 95% confidence intervals (CI) were determined via the bootstrap method (1000-resamples). 80% of the data were used in the training sets and 20% of the data in the test sets for internal validation. Beside classical prediction metrics (sensitivity, specificity, accuracy, and area under the curve (AUC)), F1-score, Cohen's kappa, and Matthews Correlation Coefficient (MCC) were calculated to test predictive performance of the model.

A sub-analysis was performed to investigate differences in specific breath feature intensities between patients on triple inhaled therapy and patients without ICS therapy. For this, raw data were obtained from a previous study published elsewhere [10], investigating the molecular breath profile of AECOPD in a cohort of patients under heterogeneous COPD treatment (no therapy, long-term mono, dual, or triple inhaled therapy). Statistical analysis was performed with R (version 4.4.1, R Core Team, Vienna, Austria).

Compound assignment

To explore the biological insights of the detected breath features and their underlying metabolic pathways, MetaboAnalyst v6.0 using the mummichog algorithm, was applied for pathway enrichment analysis (hypothesis generation) by assigning ions to potential metabolic pathways [15]. All features were arranged in ascending order based on their previously calculated p-values from paired t-testing, with the significance cut-off set at the top 10% of features. The ionisation adducts used are specified in the supplements (Table S1). The allowed mass tolerance was 2 ppm. Moreover, Refmet database [16] was used to assign breath features to putative molecules and to align them with previously detected VOCs in human breath from the literature [11, 17, 18]. Compound assignment was categorised as level 4 evidence according to Schymanski et al. [19]

Results

35 patients diagnosed with COPD hospitalised in the University Hospital Zurich because of AECOPD fulfilled the inclusion criteria and agreed to be enrolled in the study. Five patients were lost to follow-up and technical issues with measurements in two patients led to their exclusion, leaving 28 patients for the final analysis. (Fig. 1)

Detailed patient characteristics are displayed in Table 1. Among these 28 patients, 61% were male. Mean (SD) age was 68 (10.6) years. 23 of 28 patients were categorised into GOLD risk classification E, 4 patients into risk group B, and 1 patient into risk group A. Five patients (18%) had no AECOPD within the previous year, while 82% of patients had ≥ 2 moderate and/or ≥ 1 severe exacerbation within the same period. Patients had been receiving established triple inhaled therapy for a median duration of 38 months (range: 3 months to 15 years).

39% of patients had a bacterial infection, 14% had a combined viral and bacterial infection, and 43% were tested negative for both bacterial and viral infections. One patient was not tested for bacterial or viral infection.

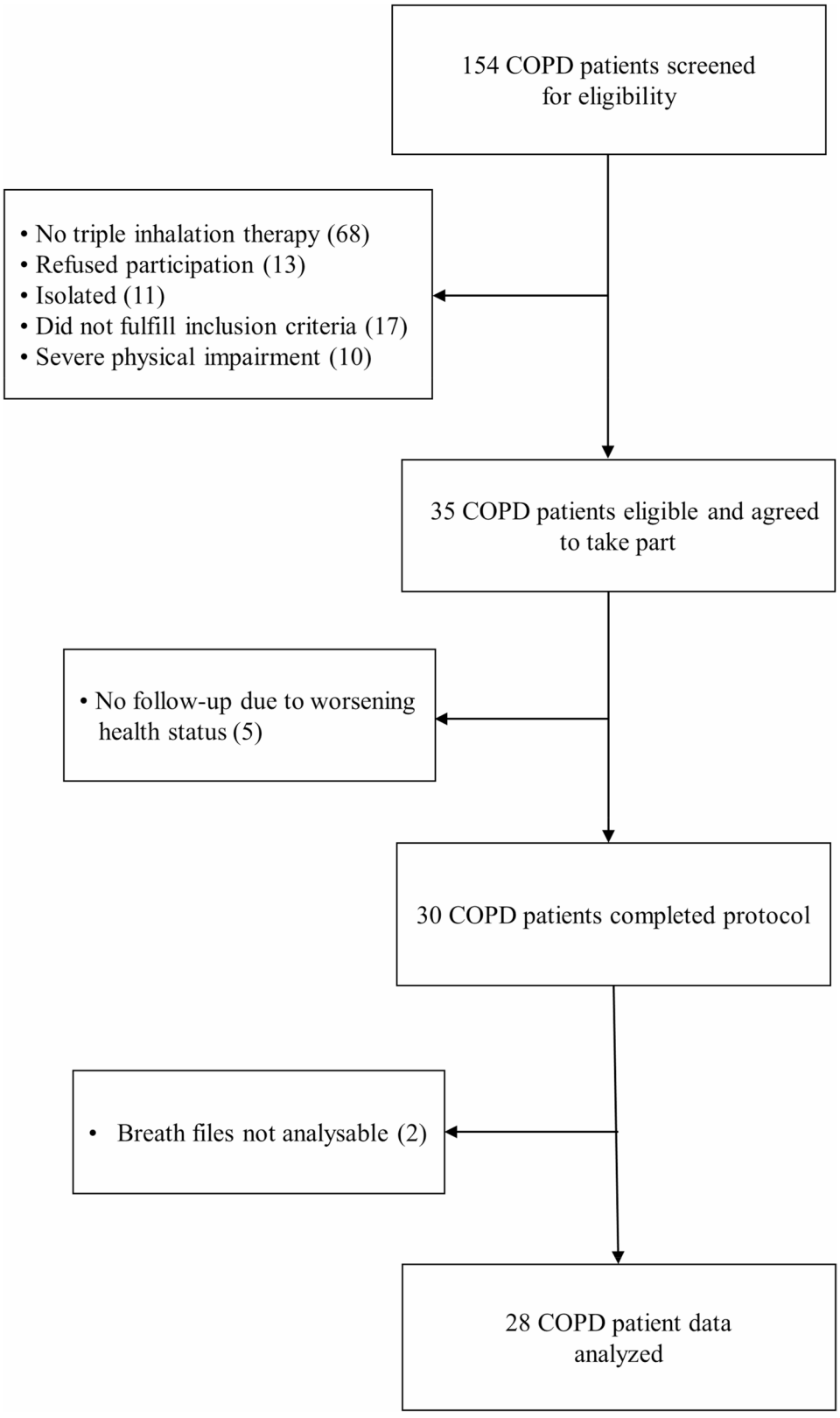

Fig. 1 Study flow

Table 1 Patient characteristics

N	28
Male/Female, N	17/11
Age, years	65 (10.2)
Pack years of smoking, N	50 (38, 80)
Smoking status	
Former smoker, N (%)	20 (71)
Never smoker, N (%)	3 (11)
Active smoker, N (%)	5 (18)
GOLD ABE Classification	
Risk category A, N (%)	1 (4)
Risk category B, N (%)	4 (14)
Risk category E, N (%)	23 (82)
Pulmonary function	
FEV1/FVC, %	44 (13.5)
FEV1, % predicted	42 (22.0)
FVC, % predicted	69 (22.2)
Exacerbation history	
Number of AECOPD previous year, N	1.3 (0.9)
Number of severe AECOPD pervious year, N	1.0 (0.7)
Treatment during AECOPD	
Antibiotics only, N (%)	4 (14)
Systemic corticosteroids only, N (%)	5 (18)
Antibiotics and systemic corticosteroids, N (%)	19 (68)
Pathogens[a]	
Bacterial only, N (%)	11 (39)
Viral only, N (%)	0 (0)
Negative, N (%)	12 (43)
Viral and bacterial, N (%)	4 (14)
Not tested, N (%)	1 (4)

Values are mean (SD) or median (25%, 75% quartiles) unless otherwise stated. [a]Microbacterial analysis of sputum culture ($N=16$), urine antigen for streptococcus pneumoniae and legionellae

pneumophila ($N=8$); comprehensive viral testing in $N=24$, COVID-19 only in $N=2$, no testing in $N=2$

N Number of patients, *AECOPD* Acute exacerbation of chronic obstructive pulmonary disease

Table 2 Clinical parameters assessed during AECOPD and subsequent stable state

	AECOPD	Stable state
Venous blood oxygen saturation, %	94.5 (2.6)	95.4 (2.3)
hs-CRP, mg/l	54 (8, 99)	3 (1, 5)
Eosinophils, G/l	0.02 (0.00, 0.19)	0.16 (0.14, 0.29)
CAT, points	23 (8)	17 (8)
mMRC, points	3 (3, 3)	2 (2, 3)
FEV1, % predicted	32 (17)	42 (22)
FeNO, ppb	29 (20, 35)	22 (12, 28)

Values are mean (SD) or median (25%, 75% quartiles) unless otherwise stated

AECOPD Acute exacerbation of COPD, *hs-CRS* high-sensitive C-reactive protein, *CAT* COPD Assessment Test, *mMRC* modified Medical Research Council Dyspnea Scale, *FEV1* Forced expiratory volume in the first second, *FeNO* Fractional exhaled nitric oxide

The predominant therapy for AECOPD was systemic corticosteroids in combination with antibiotics (68%). Median (quartiles) time between hospital admission and breath measurement was 1 (1, 2) day, while V2 was performed after a median (quartiles) of 90 (54, 171) days after V1. Clinical parameters measured during AECOPD and stable state are presented in Table 2.

Detailed patient characteristics of the previous study used for sub-analysis are described elsewhere [10]. Patient characteristics were comparable between the two studies, with 60% male participants, mean (SD) age of 65 (10.2) years, and 51% GOLD risk classification E. Long-term inhaled therapy at baseline was as follows: 20 patients (57%) received triple inhaled therapy (ICS + LAMA + LABA), eight patients (23%) received dual inhaled therapy (LAMA + LABA or short-acting muscarinic antagonist (SAMA) + short-acting beta-2 agonist (SABA)), one patient (3%) received mono inhaled therapy (LAMA), and six patients (17%) were not on any inhaled therapy. Patients not receiving triple therapy were collectively categorised as "patients not on ICS".

Metabolic pathway alterations during AECOPD

In total, intensity changes of 3298 breath features were compared between AECOPD and stable state. Pathway enrichment analysis revealed that alterations during AECOPD were predominantly associated with aminosugar, linoleate, and butanoate metabolism (see Table S2).

The aminosugars glucosamine and N-Acetylglucosamine, two building blocks of glycosaminoglycans (GAG), exhibited 4-fold and 8-fold reductions in intensities during AECOPD compared to stable state (Table 3).

In a sub-analysis comparing the current results to data from a previous study [10], N-Acetylglucosamine level was found to be 50% lower in stable state among patients receiving long-term triple inhaled therapy compared to those not on ICS. During AECOPD, N-Acetylglucosamine level dropped 6-fold in patients undergoing long-term triple inhaled therapy, while the level only dropped 1.4-fold in patients not on ICS (Table 4).

Breath features assigned to the breakdown products of the 13(s)-HPODE pathway in Linoleate metabolism showed a 2- to 4-fold decrease in intensity during AECOPD (Table S3). Breath features Linked to the butanoate metabolism showed a 2- to 4-fold change between AECOPD and stable state (Table S4). A sub-analysis of butyric acid intensity revealed that the change in butanoate metabolism seemed to be driven by a 2- to 4-fold increase in butyric acid level during stable state in patients receiving long-term triple inhaled therapy, compared to those not on ICS (Table S5).

Table 3 Breath features assigned to aminosugar metabolism

Putative chemical family	Putative molecular name	m/z	Log2FC	*p*-value	q-value	Putative formula	Ionisation
Aminosugars metabolism	Glutamic acid	130.04987	1.02	0.014	0.442	C5H9NO4	M-H2O + H [1+]
		148.0604	1.51	0.072	0.485		M + H [1+]
	Glucosamine	180.0866	2.14	0.019	0.442	C6H13NO5	M + H [1+]
		162.0761	2.40	0.046	0.461		M-H2O + H [1+]
	Pyruvic acid	106.0499	2.44	0.002	0.377	C3H4O3	M + NH4 [1+]
	N-Acetylglucosamine	204.0867	3.63	< 0.001	0.260	C8H15NO6	M-H2O + H [1+]
	N-Trimethyl-2-aminoethylphosphonate	186.11247	1.45	0.077	0.488	C5H14NO3P	M + NH4 [1+]

Breath features assigned to aminosugar metabolism as one of the most relevant altered metabolic pathways and assigned putative compounds during stable state (V2) compared to AECOPD (V1)

AECOPD Acute exacerbation of COPD, *m/z* mass-to-charge ratio, *Log2FC* Logarithm of 2 of the fold change

Table 4 N-Acetylglucosamine during AECOPD and stable state

	AECOPD	Stable state
Patients without inhaled corticosteroid therapy, previous study	2194	3104
Patients under triple inhaled therapy, previous study	276	1552
Patients under triple inhaled therapy, current study	252	1663

Intensity levels of breath feature assigned to N-Acetylglucosamine during AECOPD and stable state in patients with COPD receiving long-term triple inhaled therapy compared to patients without long-term inhaled corticosteroid therapy

AECOPD Acute exacerbation of COPD

Prediction model for AECOPD

Selecting the most discriminative breath features yielded a prediction model including 33 breath features (Table S6). A high discriminative performance between AECOPD and stable state was achieved in the training set with an AUC of 0.88 (95% CI of 0.86, 0.91), sensitivity of 81% (95% CI of 77, 86), and specificity of 82% (95% CI of 77, 86). In the testing set, the discriminative performance between AECOPD and stable state was comparable with a good AUC of 0.84 (Fig. 2), sensitivity of 86%, and specificity of 86%. Training and testing models demonstrated robust and reliable performance, with a high F1-score of 0.81 (training) and 0.86 (testing) indicating balanced precision and recall, a Cohen's kappa of 0.63 (training) and 0.71 (testing) reflecting substantial agreement beyond chance, and a MCC of 0.67 (training) and 0.71 (testing) confirming strong predictive stability across class distributions. Figure 3 shows the change in mean log2 intensity of the 33 breath features included in the prediction model.

Discussion

Despite adherence to guideline-based management, some patients with COPD treated with maximal inhaled therapy continue to experience frequent exacerbations [7]. Various reasons for this have been proposed and include underlying different exacerbation phenotypes, bacterial and viral infections, and the role of chronic systemic inflammation. However, so far it remained largely unclear why triple inhaled therapy does not effectively target the relevant inflammatory mechanisms in all patients and thus these individuals are considered non-responders to inhaled corticosteroids.

Exhaled breath analysis provides a promising tool to elucidate a broad spectrum of metabolic pathways involved in AECOPD despite triple inhaled therapy. Our results showed alterations in molecular breath features assigned to predominantly aminosugar, linoleate, and butanoate metabolism.

As airway remodeling is a key feature of COPD, previous studies have investigated the impact of extracellular matrix (ECM) turnover on disease progression and exacerbations. These studies revealed that chronic inflammation and AECOPD are associated with disturbances in the ECM turnover [20] and that GAGs, compounds of the ECM, may be useful biomarkers for both disease progression and exacerbations [21]. Papakonstantinou et al. showed that the turnover of hyaluronic acid (HA) is a major factor for the development, progression, and resolution of inflammatory lung diseases such as COPD [22, 23]. HA is a non-sulfated GAG whose biological function depends on its molecular size. Low-molecular-weight HA is produced during inflammation, has pro-inflammatory and tissue damaging properties, and interacts mainly via Toll-like receptors [24]. In contrast, high-molecular-weight HA shows anti-inflammatory and immunosuppressive properties [25]. In airway smooth-muscle cells from patients with stable COPD, HA turnover is impaired by decreased synthesis of high-molecular-weight HA and concomitant up-regulation of enzymatic degradation into low-molecular-weight HA [26]. Bronchoalveolar lavage samples demonstrated that during AECOPD, the dysregulation of HA turnover is exacerbated, resulting in elevated levels of low-molecular-weight HA [23].

N-Acetylglucosamine has various functions in the ECM, mainly as a building block of HA, as energy source of immune cells, and as structural support in mucins, the key glycoproteins that make up mucus [27–29]. Additionally, N-Acetylglucosamine plays an important role as carbon source in the energy producing hexosamine

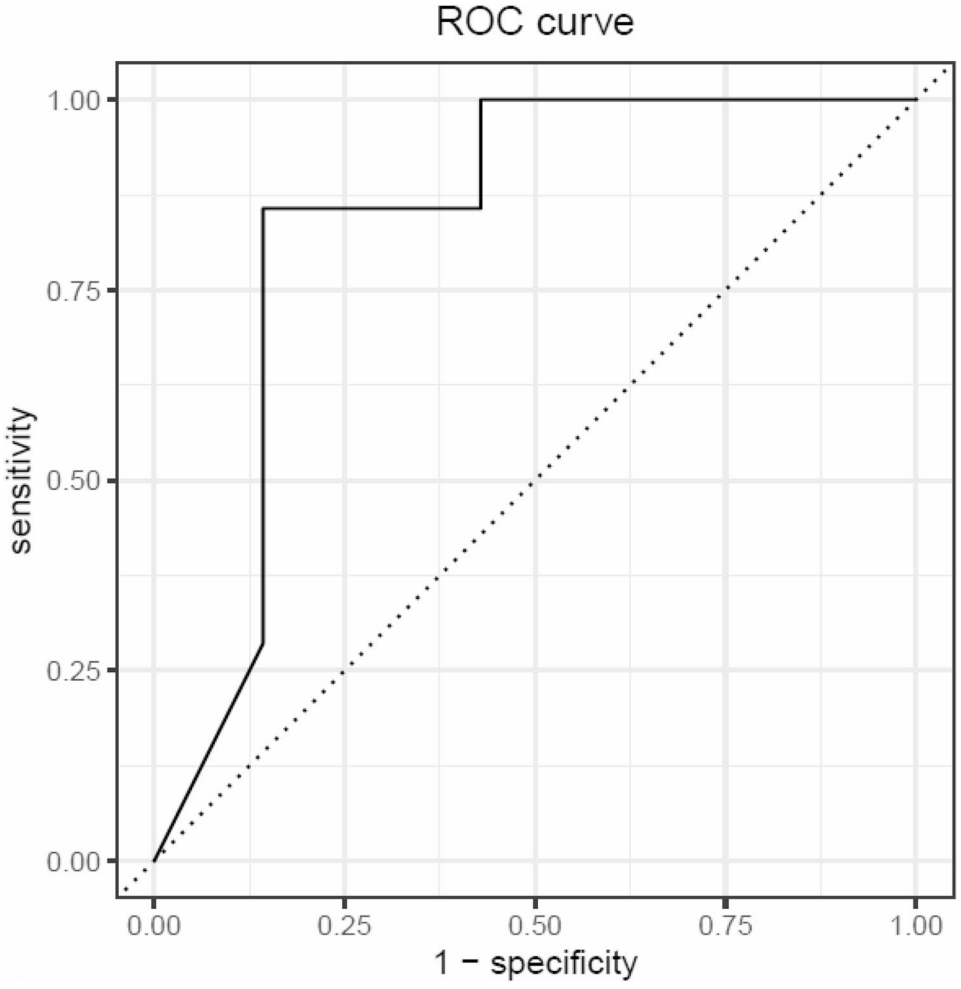

Fig. 2 ROC curve for predicting acute exacerbation of COPD (AECOPD) via exhaled breath. This ROC curve illustrates the diagnostic performance of 33 selected exhaled breath features in distinguishing AECOPD from stable state. The x-axis represents 1 - specificity (false positive rate), and the y-axis represents sensitivity (true positive rate). The solid black line represents the model's performance, while the diagonal dotted line represents the line of no discrimination (random classification). The area under the curve (AUC) is 0.84, indicating good discriminatory ability of the breath-based features in differentiating between AECOPD and stable state

biosynthesis. Hexosamine biosynthesis is enhanced in conditions where glycolysis is impaired such as in insulin resistance [30].

In a previous study investigating the effect of ICS + LABA on airway smooth muscle cells, the pathologic metabolism of HA with increased degradation of HA into low-molecular-weight HA and reduces synthesis of high-molecular-weight HA in patients with COPD was counteracted using ICS + LABA by stimulation of high-molecular-weight HA secretion and reduced enzymatic degradation into low-molecular-weight HA [31].

The sub-analysis comparing the molecular breath profile of AECOPD and stable state in a previous cohort of patients under heterogeneous COPD treatment [10] with the corresponding results in the current cohort showed a 50% reduction in N-Acetylglycosamine intensity during stable state in patients on long-term triple inhaled therapy compared to those not receiving ICS. These findings provide a possible explanation for the occurrence of further AECOPD despite triple inhaled therapy. We hypothesize that impaired effectiveness of ICS in reducing chronic inflammation is leading to a persistent chronic inflammation in our patient cohort. Thus, as the reduced levels of N-Acetylglucosamine found in our study indicate a potentially higher demand of N-Acetylglucosamine as substrate for immune cells and mucus production. This suggests that the beneficial effects of ICS + LABA in stimulating high-molecular-weight HA secretion and reducing its enzymatic degradation into low-molecular-weight HA could be absent in patients with AECOPD despite long-term triple inhaled therapy. The absence of a beneficial effect would lead to an accumulation of pro-inflammatory, low-molecular-weight HA in the stable phase, exacerbating chronic inflammation and structural damage of ECM by maintaining the innate inflammatory response, thereby increasing the risk of subsequent

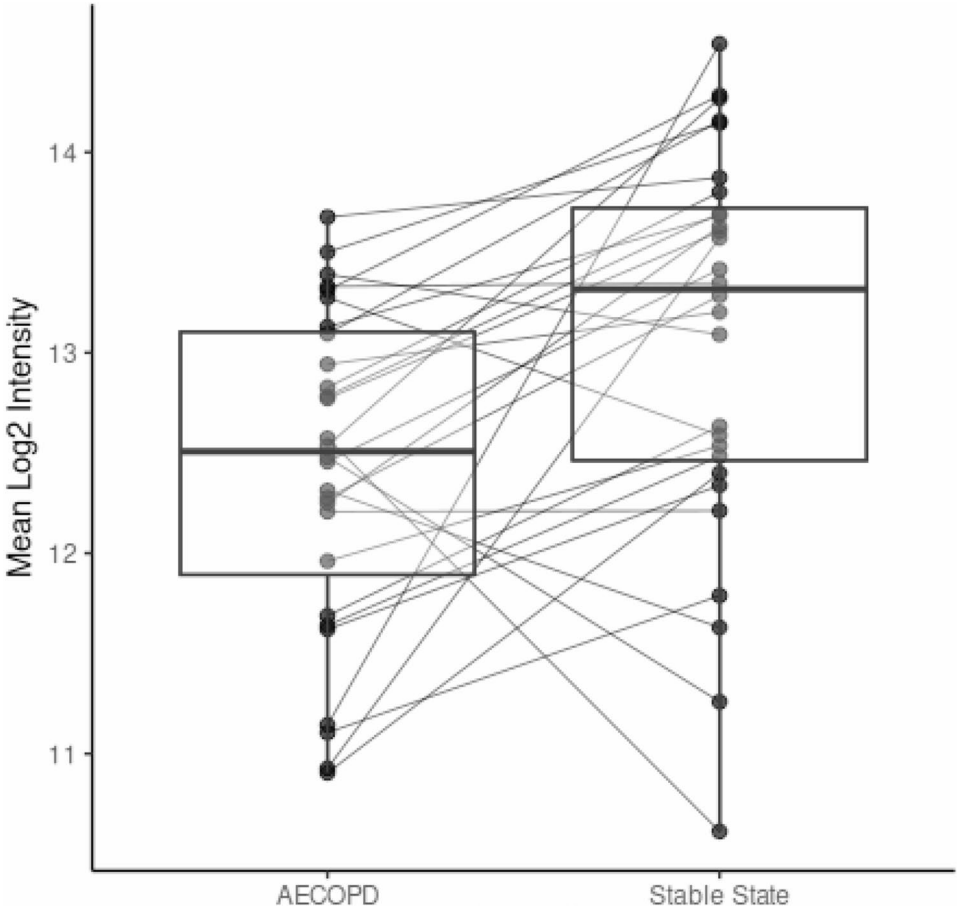

Fig. 3 Changes in breath feature intensities between AECOPD and stable state in the prediction model. Boxplots show the distribution of mean log2 intensity values of the 33 breath features selected for the prediction model during acute exacerbations of COPD (AECOPD) and at stable state. Each dot represents an individual patient, and lines connect paired measurements to illustrate within-subject changes across the two clinical states

AECOPD [20] as low-molecular-weight HA increases the expression and secretion of cytokines and chemokines involved in pulmonary inflammatory response, such as TNF-α, IL-8, and IL-6 [24]. Figure 4 illustrates this hypothesized cascade.

Further studies are needed to confirm if patients with AECOPD despite triple inhaled therapy show lower levels of N-Acetylglucosamine during stable state compared to those under triple inhaled therapy without further AECOPD, potentially indicating a non-response to ICS in relation to hyaluronic acid metabolism. If confirmed, low-molecular-weight HA could be a potential therapeutic target using immunosuppressants such as TNF-α blocker or IL-6 inhibitor to prevent ECM destruction, thus reducing exacerbation frequency [20, 24].

During AECOPD, intensity of the molecular breath feature assigned to N-Acetylglucosamine was 16-fold decreased in our study. In contrast, aminosugar metabolism measured via exhaled breath was not relevantly affected during AECOPD in a COPD cohort with patients on heterogeneous long-term inhalation therapy [10]. We hypothesise that the increased demand of N-Acetylglucosamine during AECOPD could be linked to the high energy demand of immune cells and the increased production of mucus during acute inflammation. Furthermore, long-term use of ICS could increase insulin resistance, a known adverse effect of ICS [32], promoting the use of N-Acetylglucosamine as alternative energy source for the cells. This hypothesis is supported by the results of our sub-analysis which revealed a remarkably higher decrease in N-Acetylglucosamine during AECOPD compared to stable state in patients on long-term triple inhaled therapy compared to those not on ICS.

Linoleate and butanoate metabolism were identified as an additional altered metabolic pathway between AECOPD and stable state. Linoleic acid, the most abundant polyunsaturated fatty acid (PUFA) in humans, is metabolised into lipid mediators such as pro-inflammatory leukotrienes and prostaglandins through the arachidonic acid pathway [33]. A study examining induced sputum from patients with COPD and smoking controls

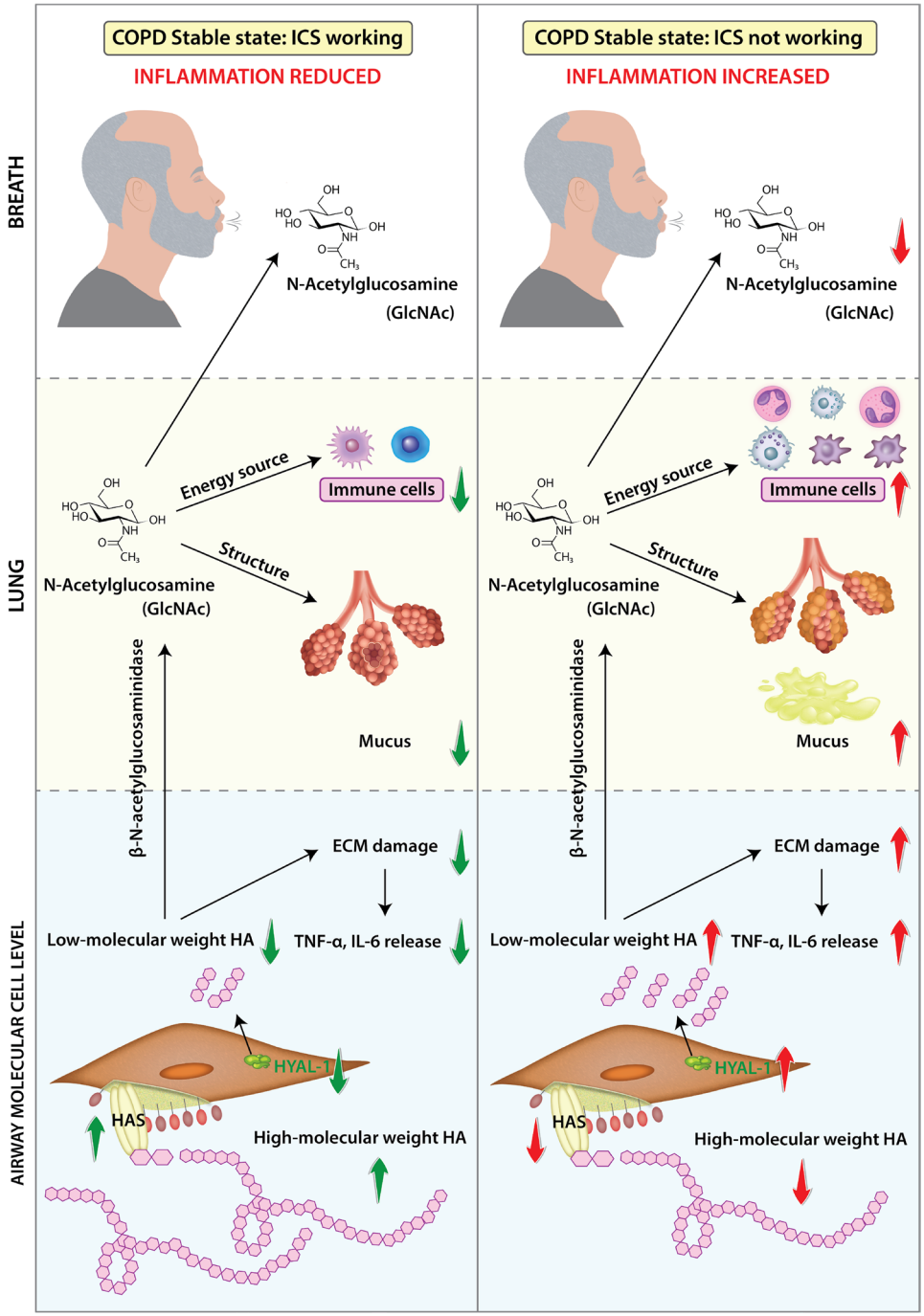

Fig. 4 Role of N-Acetylglucosamine (GlcNAc) in COPD under inhaled corticosteroid (ICS) therapy. This schematic illustrates the molecular and cellular changes hypothesized to be associated with COPD in a stable state when ICS therapy is effective (left) versus when ICS therapy is ineffective (right). At the top (breath level): in stable COPD with effective ICS therapy, the presence of putative N-GlcNAc in the breath is higher, correlating with reduced inflammation. In contrast, when ICS therapy is ineffective, GlcNAc levels in the breath decrease, accompanied by increased inflammation. In the middle (lung level): GlcNAc serves as an energy source and structural component for lung cells. In an ICS-responsive state, immune cell activation and mucus production are reduced. Conversely, ICS resistance results in excessive immune cell recruitment and increased mucus secretion, contributing to airway obstruction. At the bottom (airway molecular cell level): At the molecular level, hyaluronan synthase (HAS) and hyaluronidase (HYAL-1) activity regulate extracellular matrix (ECM) homeostasis. Under effective ICS therapy, high-molecular-weight hyaluronic acid (HA) is preserved, while low-molecular-weight HA and inflammatory cytokines (such as TNF-α, IL-6) are reduced, leading to less ECM damage. In ICS-resistant states, a decrease in HAS and an increase in HYAL-1 activity lead to increased levels of low-molecular weight HA and reduced levels of high-molecular weight HA, resulting in excessive ECM degradation, increased pro-inflammatory cytokine release, and heightened inflammation *Green arrows indicate a healthy response, demonstrating reduced inflammation when corticosteroids are effective. Red arrows signify an unhealthy response, representing persistent inflammation when ICS fail to work*

revealed decreased levels of linoleic acid in individuals with stable COPD [34]. Based on our finding that metabolites assigned to the Linoleic acid pathway are reduced during AECOPD, we propose that Linoleate predominantly contributes to the inflammatory processes via the arachidonic acid pathway, leading to a reduction in metabolites associated with other pathways such as 13(s)-HPODE within the linoleate metabolism. This finding seems to represent the acute inflammation response during AECOPD and is in line with the results from a previous study investigating molecular changes in exhaled breath during AECOPD in a cohort of patients with COPD on heterogeneous long-term inhaled therapy showing linoleate metabolism as a predominantly affected metabolic pathway [10].

Butyrate is a short-chain fatty acid (SCFA) predominantly produced in the large intestine following anaerobic fermentation of soluble dietary fibers by gut microbiota. SCFA are proposed as a key link in the metabolic and immune axis between the gut and lungs [35]. The effects of butyrate in the lungs appear to occur indirectly by modulating immune cell function [36]. Lower overall levels of SCFAs were found in COPD patients compared to healthy controls [37]. We found decreased levels of metabolites assigned to butanoate metabolism during AECOPD compared to stable state. However, the mechanisms through which butyrate influences immune cells to contribute to the inflammatory response in COPD remain unclear. A sub-analysis of butyric acid intensity revealed that the change in butanoate metabolism seemed to be driven by an increase in butyric acid level during stable state in patients receiving long-term triple inhaled therapy, compared to those not on ICS. Therefore, butanoate and linoleate metabolism seem to provide less a therapeutic approach for preventing AECOPD despite triple inhaled therapy.

In addition to exploring underlying metabolic alterations, exhaled breath analysis facilitates the development of identification models for AECOPD by identifying distinct molecular breath features associated with key metabolic pathways. Our model, which included 33 molecular breath features, achieved an AUC of 0.87, reflecting good discriminative ability in distinguishing AECOPD from stable state. Sensitivity of 82% ensures reliable detection of patients with AECOPD, minimizing the risk of missed diagnoses, while the specificity of 82% improves prevention of unnecessary use of systemic corticosteroids and/or antibiotics. The combination of high discriminative ability with balanced, high sensitivity and specificity highlights the model's potential for integration into clinical workflows, improving diagnostic accuracy for AECOPD in a non-invasive manner within minutes. Our findings emphasize the need for continued research in exhaled breath analysis to identify biomarkers and metabolic pathways underlying AECOPD, and AECOPD despite triple inhaled therapy. In particular, longitudinal sampling during the steady state would provide important information on reproducibility and temporal stability of breath-based signals. Further studies on the chemical structure of specific breath metabolites associated with these pathways will facilitate the ultimate identification of metabolic changes during AECOPD.

A limitation of this study is that chemical identification of the altered metabolites and associated metabolic pathways was inferred through database matching of measured accurate masses (within 2ppm). Definitive identification of molecules remains an important challenge in exhaled breath analysis, requiring further investigations using advanced techniques such as ultra-performance liquid chromatography–MS/MS analysis with chemical standards.

Conclusion

Investigating metabolic changes during AECOPD compared to stable state in patient with exacerbations despite triple inhaled therapy provided disturbances in amino-sugar metabolism as a potential underlying mechanism and therapeutic target. Moreover, non-invasive, real-time exhaled breath analysis offers the potential to diagnose AECOPD within minutes, which could enhance diagnostic accuracy and treatment efficiency of AECOPD in clinical settings.

Abbreviations

AECOPD	Acute exacerbation of chronic obstructive pulmonary disease
AUC	Area under the curve
CAT	Chronic obstructive pulmonary disease assessment test
CI	Confidence interval
COPD	Chronic obstructive pulmonary disease
DBI	Deep breath intelligence ag
ECM	Extracellular matrix
FeNO	Fractional exhaled nitric oxide
FEV1	Forced expiratory volume in the first second
FVC	Forced vital capacity
GAG	Glycosaminoglycans
GOLD	Global initiative for chronic obstructive lung disease
HA	Hyaluronic acid
Hs-CRP	High-sensitive C-reactive protein
ICS	Inhaled corticosteroid
LAMA	Long-acting muscarinic antagonist
LABA	Long-acting beta-agonist
log2FC	Log 2 - Fold change
MDLsmp	Minimum description length model
mMRC	Modified medical research council dyspnea scale
m/z	Mass-to-charge ratio
PUFA	Polyunsaturated fatty acid
SAMA	Short-acting muscarinic antagonist
SABA	Short-acting beta-2 agonist
SCFA	Short-chain fatty acid
SD	Standard deviation
SESI-HRMS	Secondary electrospray ionization - high-resolution mass spectrometry
V1 and V2	Visit 1 and visit 2
VOCs	Volatile organic compounds

Supplementary Information

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Supplementary Material 1.

Authors' contributions

Conceptualization: MK; methodology: MK; formal analysis and investigation: NAS, FS, SB, KF, JH, DMB; draft of the manuscript: NAS; critical revision of the manuscript for important intellectual content: FS, SB, KF, JH, DMB, MK; funding acquisition: MK.

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Data availability

The data that support the findings of this study are available on reasonable request from the corresponding author.

Declarations

Ethical approval and consent to participate

The study was approved by the cantonal ethics committee of Zurich (BASEC-Nr. 2020 – 01954) and all participants provided written informed consent.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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References

1. Berry CE, Wise RA. Mortality in COPD: causes, risk factors, and prevention. COPD: Journal of Chronic Obstructive Pulmonary Disease. 2010;7(5):375–82.
2. Lieberman D, Shmarkov O, Gelfer Y, et al. Prevalence and clinical significance of fever in acute exacerbations of chronic obstructive pulmonary disease. Eur J Clin Microbiol Infect Dis. 2003;22(2):75–8.
3. O'Donnell DE, Parker CM. COPD exacerbations. 3: Pathophysiology Thorax. 2006;61(4):354–61.
4. Miravitlles M, Ferrer M, Pont A, et al. Effect of exacerbations on quality of life in patients with chronic obstructive pulmonary disease: a 2 year follow up study. Thorax. 2004;59(5):387–95.
5. Anzueto A. Impact of exacerbations on COPD. Eur Respir Rev. 2010;19(116):113–8.
6. Zayed Y, Barbarawi M, Kheiri B, et al. Triple versus dual inhaler therapy in moderate-to-severe COPD: a systematic review and meta-analysis of randomized controlled trials. Clin Respir J. 2019;13(7):413–28.
7. Chen S, Small M, Lindner L, Xu X. Symptomatic burden of COPD for patients receiving dual or triple therapy. Int J Chron Obstruct Pulmon Dis. 2018;13:1365–76.
8. Wallace MAG, Pleil JD. Evolution of clinical and environmental health applications of exhaled breath research: review of methods and instrumentation for gas-phase, condensate, and aerosols. Anal Chim Acta. 2018;1024:18–38.
9. Gaugg MT, Nussbaumer-Ochsner Y, Bregy L, et al. Real-time breath analysis reveals specific metabolic signatures of COPD exacerbations. Chest. 2019;156(2):269–76.
10. Basler S, Sievi NA, Schmidt F, et al. Molecular breath profile of acute COPD exacerbations. J Breath Res. 2024. https://doi.org/10.1088/1752-7163/ad9ac4.
11. Gisler A, Singh KD, Zeng J, et al. An interoperability framework for multicentric breath metabolomic studies. iScience. 2022;25(12):105557.
12. Singh KD, Tancev G, Decrue F, et al. Standardization procedures for real-time breath analysis by secondary electrospray ionization high-resolution mass spectrometry. Anal Bioanal Chem. 2019;411(19):4883–98.
13. Storey JD. A direct approach to false discovery rates. J R Stat Soc Series B Stat Methodol. 2002;64(3):479–98.
14. Kononenko I, editor. editor The minimum description length based decision tree pruning. Berlin, Heidelberg: Springer; 1998.
15. Pang Z, Chong J, Zhou G, et al. MetaboAnalyst 5.0: narrowing the gap between Raw spectra and functional insights. Nucleic Acids Res. 2021;49(W1):W388–96.
16. The Metabolomics Workbench. RefMet: A Reference list of Metabolite names Available from: https://www.metabolomicsworkbench.org/databases/refmet/index.php. Accessed 03.04.2025.
17. Drabińska N, Flynn C, Ratcliffe N, et al. A literature survey of all volatiles from healthy human breath and bodily fluids: the human volatilome. J Breath Res. 2021. https://doi.org/10.1088/1752-7163/abf1d0.
18. Moura PC, Raposo M, Vassilenko V. Breath volatile organic compounds (VOCs) as biomarkers for the diagnosis of pathological conditions: a review. Biomed J. 2023;46(4):100623.
19. Schymanski EL, Jeon J, Gulde R, et al. Identifying small molecules via high resolution mass spectrometry: communicating confidence. Environ Sci Technol. 2014;48(4):2097–8.
20. Postma DS, Timens W. Remodeling in asthma and chronic obstructive pulmonary disease. Proc Am Thorac Soc. 2006;3(5):434–9.
21. Caird R, Williamson M, Yusuf A, et al. Targeting of glycosaminoglycans in genetic and inflammatory airway disease. Int J Mol Sci. 2022. https://doi.org/10.3390/ijms23126400.
22. Papakonstantinou E, Bonovolias I, Roth M et al. Serum levels of hyaluronic acid are associated with COPD severity and predict survival. Eur Respir J. 2019;53(3):1801183.
23. Papakonstantinou E, Roth M, Klagas I, et al. COPD exacerbations are associated with proinflammatory degradation of hyaluronic acid. Chest. 2015;148(6):1497–507.
24. Hoarau A, Polette M, Coraux C. Lung hyaluronasome: involvement of low molecular weight Ha (Lmw-Ha) in innate immunity. Biomolecules. 2022. https://doi.org/10.3390/biom12050658.
25. Papakonstantinou E, Karakiulakis G. The 'sweet' and 'bitter' involvement of glycosaminoglycans in lung diseases: pharmacotherapeutic relevance. Br J Pharmacol. 2009;157(7):1111–27.
26. Klagas I, Goulet S, Karakiulakis G, et al. Decreased hyaluronan in airway smooth muscle cells from patients with asthma and COPD. Eur Respir J. 2009;34(3):616–28.
27. Moussian B. The role of GlcNAc in formation and function of extracellular matrices. Comp Biochem Physiol B Biochem Mol Biol. 2008;149(2):215–26.
28. Qiang A, Slawson C, Fields PE. The role of O-GlcNAcylation in immune cell activation. Front Endocrinol (Lausanne). 2021;12:596617.
29. Chatterjee M, van Putten JPM, Strijbis K. Defensive properties of mucin glycoproteins during respiratory infections-relevance for SARS-CoV-2. mBio. 2020. https://doi.org/10.1128/mBio.02374-20.
30. Paneque A, Fortus H, Zheng J, et al. The hexosamine biosynthesis pathway: regulation and function. Genes (Basel). 2023. https://doi.org/10.3390/genes14040933.
31. Papakonstantinou E, Klagas I, Karakiulakis G, et al. Glucocorticoids and beta(2)-agonists regulate the pathologic metabolism of hyaluronic acid in COPD. Pulm Pharmacol Ther. 2018;48:104–10.
32. Egbuonu F, Antonio FA, Edavalath M. Effect of inhaled corticosteroids on glycemic status. Open Respir Med J. 2014;8:101–5.
33. Galano JM, Lee YY, Oger C, et al. Isoprostanes, neuroprostanes and phytoprostanes: an overview of 25years of research in chemistry and biology. Prog Lipid Res. 2017;68:83–108.
34. van der Does AM, Heijink M, Mayboroda OA, et al. Dynamic differences in dietary polyunsaturated fatty acid metabolism in sputum of COPD patients and controls. Biochimica et Biophysica Acta (BBA). 2019;1864(3):224–33.
35. Kotlyarov S. Role of short-chain fatty acids produced by gut microbiota in innate lung immunity and pathogenesis of the heterogeneous course of chronic obstructive pulmonary disease. Int J Mol Sci. 2022. https://doi.org/10.3390/ijms23094768.

36. Correa RO, Castro PR, Moser R, et al. Butyrate: connecting the gut-lung axis to the management of pulmonary disorders. Front Nutr. 2022;9:1011732.
37. Li N, Dai Z, Wang Z, et al. Gut microbiota dysbiosis contributes to the development of chronic obstructive pulmonary disease. Respir Res. 2021;22(1):274.

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