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Molecular breath profile of acute COPD exacerbations

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Abstract

Acute exacerbations of chronic obstructive pulmonary disease (AECOPD) show high variability in individual susceptibility and promote disease progression; thus, accurate diagnosis and treatment is essential. Unravelling the molecular metabolic changes during AECOPD in breath could promote understanding of AECOPD and its treatment. Our objective was to investigate the metabolic breath profiles during AECOPD for biomarker detection. We conducted real-time breath analysis in patients with COPD during AECOPD and during subsequent stable phase. Molecular breath patterns were compared between AECOPD and stable phase by dimension reduction techniques and paired t-tests. Pathway enrichment analyses were performed to investigate underlying metabolic pathways. Partial least-squares discriminant analysis and XGboost were utilised to build a prediction model to differentiate AECOPD from stable state. 35 patients (60% male) with a mean age of 65 (10.2) yr with AECOPD were included. AECOPD could be predicted with a high sensitivity of 82.5% (95% confidence interval of 68.8%–93.8%) and an excellent discriminative power (AUC = 0.86). Metabolic changes in the linoleate, tyrosine, and tryptophan pathways during AECOPD were predominant. Significant metabolic changes occur during COPD exacerbations, predominantly in the linoleate, tyrosine, and tryptophan pathways, which are all linked to inflammation. Real-time exhaled breath analysis enables a good prediction of AECOPD compared to stable state and thus could enhance precision of AECOPD diagnosis and efficacy in clinical practice.

Abbreviations

AECOPD	Acute exacerbation of chronic obstructive pulmonary disease
AUC	Area under the curve
CI	Confidence interval
COPD	Chronic obstructive pulmonary disease
DBI	Deep breath intelligence AG
Hs-CRP	High-sensitive C-reactive protein
log2FC	Log 2-fold chance
m/z	Mass-to-charge ratio
PCA	Principal component analysis
PLS-DA	partial least-squares discriminant analysis
PUFAs	Polyunsaturated fatty acid
SD	Standard deviation
SESI-HRMS	Secondary electrospray ionization-high-resolution mass spectrometry
V1 and V2	Visit 1 and Visit 2
VIP	Variable importance projection
VOCs	Volatile organic compounds
XGboost	Extreme gradient boosting

1. Introduction

AECOPD pose a significant threat to patients with COPD, leading to a sudden decline in their quality of life, an elevated risk of hospitalization, and even death [1]. The likelihood of AECOPD rises as COPD progresses, and each exacerbation further accelerates this progression [2]. Viral or bacterial infections contribute to 50%–70% of AECOPD episodes, with environmental pollution accounting for about 10% [3]. Despite the critical importance of early and accurate treatment in influencing the course of exacerbations [4, 5], approximately 30% of AECOPD episodes have an unknown origin [6]. As specific biomarkers for AECOPD remain unidentified, the condition is currently defined by the worsening of respiratory symptoms beyond daily variations, including sudden decline in lung function, increased cough, increased sputum production, and acute shortness of breath [7]. Standard treatments for AECOPD involve intensified inhalation, systemic corticosteroids, and/or antibiotics [8], but the absence of biomarkers contributes to diagnostic uncertainty, potentially resulting in overuse of systemic corticosteroids and antibiotics.

Exhaled breath analysis emerges as a promising tool for biomarker detection, providing a non-invasive, real-time reflection of metabolic and biochemical processes in the body [9]. Recent studies employing exhaled breath analysis have identified metabolic differences between patients with stable COPD and healthy persons [10]. Additionally, patients with COPD experiencing frequent exacerbations exhibit distinct molecular breath patterns compared to patients with COPD who do not have frequent exacerbations, mostly in pathways related to inflammatory processes and oxidative stress [11]. A few single breath compounds that could discriminate AECOPD from stable COPD and healthy individuals were postulated; however, those studies were not able to identify the sources of these metabolites. Additional investigation is required to fully comprehend the molecular changes in breath patterns during AECOPD and to facilitate the identification of potential biomarkers.

Our objective was to identify putative exhaled breath biomarkers for AECOPD in a longitudinal setting, utilising real-time mass spectrometry (SESI-HRMS). For this purpose, we investigated the exhaled air of patients with COPD during AECOPD and subsequently during a stable state.

2. Study design and methods

2.1. Study design and subjects

This prospective observational study, conducted at the University Hospital Zurich between January 2020 and September 2022, focused on hospitalised individuals with COPD experiencing AECOPD, and included both female and male patients. Inclusion criteria comprised a minimum age of 18 yr, a pre-existing diagnosis of COPD, and admitted with clinically diagnosed AECOPD. Patients who could not give informed consent or follow the protocol due to physical or intellectual limitations were excluded. Additionally, those receiving intensive clinical monitoring, those under contact isolation, pregnant individuals, and those with unstable endocrine conditions (such as poorly controlled diabetes mellitus) were also excluded. Patients underwent two study visits. The initial V1 occurred during the acute exacerbation phase (within 72 h after hospital admission), while the second V2 took place after a recovery period of at least 8 weeks at a time when patients were expected to have returned to a stable COPD phase. During both visits, patients were asked to provide information about their recent consumption of food, beverages, caffeine, cigarettes, alcohol, cosmetics, smoking history, comorbidities, and medication intake.

Hospital records were consulted to confirm clinical diagnosis of AECOPD and medical history. Inflammatory markers, including Hs-CRP and blood leukocyte levels, microbiological data, blood oxygen saturation level, and duration of hospitalization were collected. The study was approved by the cantonal ethics committee of Zurich (BASEC-Nr. 2019–000 30), and adhered to the principles of the Declaration of Helsinki and Good Clinical Practice. The study was registered at ClinicalTrials.gov (NCT05456009) and all participants provided written informed consent.

2.2. Breath analysis

A Secondary Electrospray Ionization source (Fossil Ion Technology, Spain) coupled to Q Exactive Plus high-resolution mass spectrometry (Thermo Fisher Scientific, Germany) (SESI-HRMS) was used for online breath analysis. Room temperature was maintained at a constant level. The breath analysis protocol has been described previously [12, 13]. In short, participants exhaled through a sterile filter attached to a heated tube (made of stainless steel and coated with Silconert) measuring 50 cm in length and 4 mm in diameter, linked to the curtain AUX gas port of an Orbitrap spectrometer. To prevent water condensation and loss of compounds on the tube walls, an insulated heating tape was set at 130 °C. The volume in the sampling line was continuously monitored by a digital capnograph throughout the entire exhalation process. Breath samples were logged in real time and six repetitions were carried out in both positive and negative modes.

2.3. Spectra analysis

The initial dataset consisted of 140 mass spectrometer files and capnograph files (70 files from AECOPD and 70 files from the stable COPD phase), which underwent preprocessing using a pipeline developed by (DBI, Switzerland). Utilizing proprietary software built upon Thermo Fisher Scientific's RawFileReader, the mass spectral data were directly extracted from the RAW files. The resulting data matrix comprised 2137 features, encompassing 1350 (m/z) in positive mode and 787 in negative mode.

2.4. Statistics

To examine variations in feature intensities between V1 and V2, fold-change values were calculated by logarithmically transforming the ratio of feature signal intensity in V1 to V2 (log2FC). Differences in feature intensities between V1 and V2 were evaluated using paired t-tests. P-values were adjusted for multiple comparison by computing their corresponding q-values [14].

The prediction model was built by PLS-DA for dimensionality reduction, using the criterion of the lowest prediction error in cross validation (random subsets). Components were further used as input for an model (XGboost). Breath features with a VIP score $\geqslant 1$ in the testing model were included in the final model. 100-fold cross-validation were computed and the 95% CIs were determined via the bootstrap method (2000-resamples). 80% of the data were used in the training sets and 20% of the data in the test sets.

Statistical analysis was performed with R (version 4.4.1, R Core Team, Vienna, Austria).

2.5. Compound assignment

To elucidate the biological implications of the measured breath features, two methods were applied to assign exact mass features to potential molecular compounds: (i) Utilising DBI's proprietary SESI-HRMS database, molecular formulas were assigned following the golden rules for heuristic filtering outlined by Fiehn and Kind [15]; (ii) MetaboAnalyst v6.0, using the mummichog algorithm, was applied for pathway enrichment analysis to add more biological insights (hypothesis generation) by translating ions to potential metabolic pathways [16]. All features were arranged in ascending order based on their previously calculated p-values from a paired t-test, with the significance cut-off set at the top 10% of features. The ionization adducts used are specified in the supplements (table (S1)). The allowed mass tolerance was 2 ppm. We denote our evidence level of compound assignment at level 4 according to Schymanski *et al* [17].

3. Results

54 patients diagnosed with COPD were admitted to the University Hospital Zurich for AECOPD and underwent eligibility screening. Out of these, 36 patients fulfilled the inclusion criteria and were successfully enrolled in the study (figure (S1)). One patient was lost to follow-up, leading to the final analysis involving 70 breath measurement files from 35 patients.

Among these 35 patients, mean (SD) age was 65 (10.2) yr and 60% were male. 20 of the 35 patients (57.1%) were former smokers, while 13 patients (37.1%) were active smokers. The mean (SD) pack years smoked was 48 (27). 57.2% of the patients had severe (GOLD III) to very severe (GOLD IV) airflow obstruction. Eight out of 35 patients had a bacterial infection, one had a viral infection, seven had a combined infection (viral and bacterial), 16 were tested negative for bacterial and viral infection, and three were not tested. The predominant therapy for AECOPD was systemic corticosteroids (88.6%). Median (quartiles) length of stay was 7 (4.5–9.0) d (table 1).

3.1. Breath alterations during AECOPD and prediction

Table (S2) shows the detected breath features with their intensity changes (log2FC) during AECOPD and the statistical significance. Pathway enrichment analysis revealed that the alterations during AECOPD of the 10% top breath features were predominantly associated with the linoleate, tyrosine, and tryptophan metabolism (see tables 2 and (S3)).

PLS-DA revealed that combining the first eight PLS-DA components in the prediction model is associated with the lowest classification error rate. The prediction model includes 1437 breath features and revealed good sensitivity of 82.5% (95% CI of 68.8%–93.8%), good specificity of 77.1% (95% CI of 63.3%–90.3%), and good accuracy of 79.8% (95% CI of 70.0–88.6%) to predict AECOPD compared to a stable state. The AUC showed an excellent overall discriminative power of 0.86 (95% CI of 0.77–0.94). Figure 1 shows the cross-validated PLS-DA score plot for the comparison of AECOPD and stable state.

Table (S4) displays the 1437 breath features included in the prediction model with the corresponding component scores. The most discriminative breath features out of the prediction model are depicted in a volcano plot illustrating the corresponding log2FC and the statistical significance (figure 2).

4. Discussion

Considerable efforts have been devoted to exploring the metabolome of patients with COPD, mostly driven by the incomplete understanding of various aspects of the disease. These efforts include the understanding of diverse disease trajectories and the susceptibility to AECOPD. The advancement of increasingly precise measurement techniques presents new avenues to unravel the characteristics of the disease.

Table 1. Patient characteristics.

N	35
Female sex, *N* (%)	14 (40.0)
Age, years	65 (10.2)
Airflow obstruction grades according to GOLD, *N* (%)	
1	5 (14.3)
2	10 (28.6)
3	10 (28.6)
4	10 (28.6)
Pack years of smoking, *N*	48 (27.0)
Smoking status, *N* (%)	
Former smoker, *N* (%)	20 (57.1)
Never smoker, *N* (%)	2 (5.7)
Active smoker, *N* (%)	13 (37.1)
COPD risk category	
Undefined, *N* (%)	1 (2.9)
Risk category A, *N* (%)	8 (22.9)
Risk category B, *N* (%)	8 (22.9)
Risk category E, *N* (%)	18 (51.4)
Treatment during AECOPD	
Only antibiotics, *N* (%)	4 (11.4)
Only systemic corticosteroids, *N* (%)	13 (37.2)
Antibiotics and systemic corticosteroids, *N* (%)	18 (51.4)
Pathogen	
Bacterial, *N* (%)	8 (22.9)
Viral, *N* (%)	1 (2.9)
Negative, *N* (%)	16 (45.7)
Viral and bacterial, *N* (%)	7 (20.0)
Not tested, *N* (%)	3 (8.6)
Hospitalization duration, days	7.0 [4.5, 9.0]

Values are mean (SD) or median (25%/75% quartiles) unless otherwise stated. *N*: number of patients.

Particularly crucial are the frequency and severity of AECOPD, as severe exacerbations accelerate disease progression and increase mortality [1]. Furthermore, the discovery of biomarkers for AECOPD is an important endeavour as the current diagnostic uncertainty hampers early detection and diagnosis and may therefore delay targeted treatment or may result in overtreatment.

Despite the recognised importance of discovering diagnostic biomarkers for AECOPD and the numerous studies focusing primarily on blood-based biomarkers, the translation of these predominantly single biomarkers into clinical practice has not occurred due to challenges in accurately and consistently diagnosing AECOPD [18]. Thus, elucidating the various metabolic pathways affected by COPD and the metabolic changes present during AECOPD is a much desired approach. Previous studies on biospecimens such as blood, urine, lung tissue, or exhaled breath condensate analysed the metabolic pathways affected by COPD [19]. For example, Van Berkel *et al* [20]. Discerned patients with COPD from non-diseased controls based on the presence of specific VOCs in their breath. Additionally, Gaugg and colleagues [11] identified sustained alterations during exacerbation-free intervals in the breath profiles of patients experiencing frequent COPD exacerbations compared to those without frequent exacerbations. In patients predisposed to frequent exacerbations, the ω-oxidation pathway was downregulated and specific nitro-aromatic metabolites were upregulated in exacerbation-free intervals, suggesting sustained disruptions in oxidative stress pathways and inflammatory processes. These features related to the ω-oxidation pathway were also found with a downregulated trend, suggesting a consistent alteration in COPD patients with known exacerbations (table (S2)).

Importantly, the present analysis of metabolic alterations during an acute exacerbation phase suggests a predominant activation of the linoleate, tryptophan, and tyrosine pathways. The reduced levels of multiple metabolites of the compromised tyrosine pathway during AECOPD might serve as potential biomarkers for AECOPD. Such reduced levels of tyrosine are recognised as a result of increased oxidative stress and inflammation, both prevalent in AECOPD [21].

Table 2. Most relevant altered metabolic pathways and assigned putative compounds during AECOPD (V1) vs stable state (V2).

Putative chemical family	Putative molecular name	m/z	Log2FC	p-value	q-value	Putative formula	Ionisation
Linoleate metabolism	trans-4,5-epoxy-(2E)-decenal	169.122 31	−0.59	0.006	0.021	C10H16O2	M + H [1+]
		170.12566	–0.55	0.025	0.042		M(C13)+ H [1+]
		186.14886	−2.14	<0.001	0.003		M + NH4 [1+]
	3,4-epoxynonanal; 4-hydroxynonenal	174.148 86	−2.12	0.006	0.020	C9H16O2	M + NH4 [1+]
Tryptophan metabolism	unknown	134.096 43	−1.42	0.007	0.115	C9H11N	M + H [1+]
	3-hydroxyanthranilate	−153.038 68	1.61	0.009	0.024	C7H7O3N	M(C13)−H [1−]
		–152.035 32	0.43	0.021	0.038		M−H [1−]
	2-amino-3-carboxymuconate semialdehyde	−184.025 14	0.99	0.006	0.020	C7H7O5N	M−H [1-]
Tyrosine metabolism	L-Adrenaline; L-Normetanephrine; Norsalsolinol; L-Phenylalanine	166.086 24	−0.70	0.023	0.121	C9H13 NO3	M−H2O + H [1+] M + H [1+]
	(−)-Salsoline;	194.117 57	−0.75	0.009	0.115	C11H15 NO2	M + H [1+]
	(S)-N-Methylsalsolinol	176.106 98	–1.04	0.022	0.121		M−H2O + H [1+]
	Hordenine	166.122 63	−1.30	0.001	0.012	C10H15ON	M + H [1+]
	N-Methyltyramine	134.096 43	−1.42	0.007	0.115	C9H13NO	M-H2O + H [1+]
	Epinine; 3-Methoxytyramine	168.101 91	−0.86	0.011	0.115	C9H13NO2	M + H [1+]

Putative formula are at an evidence level 4 by Schymanski *et al* [17]. AECOPD: acute exacerbation of COPD; m/z; Log2FC: logarithm of 2 of the fold change.

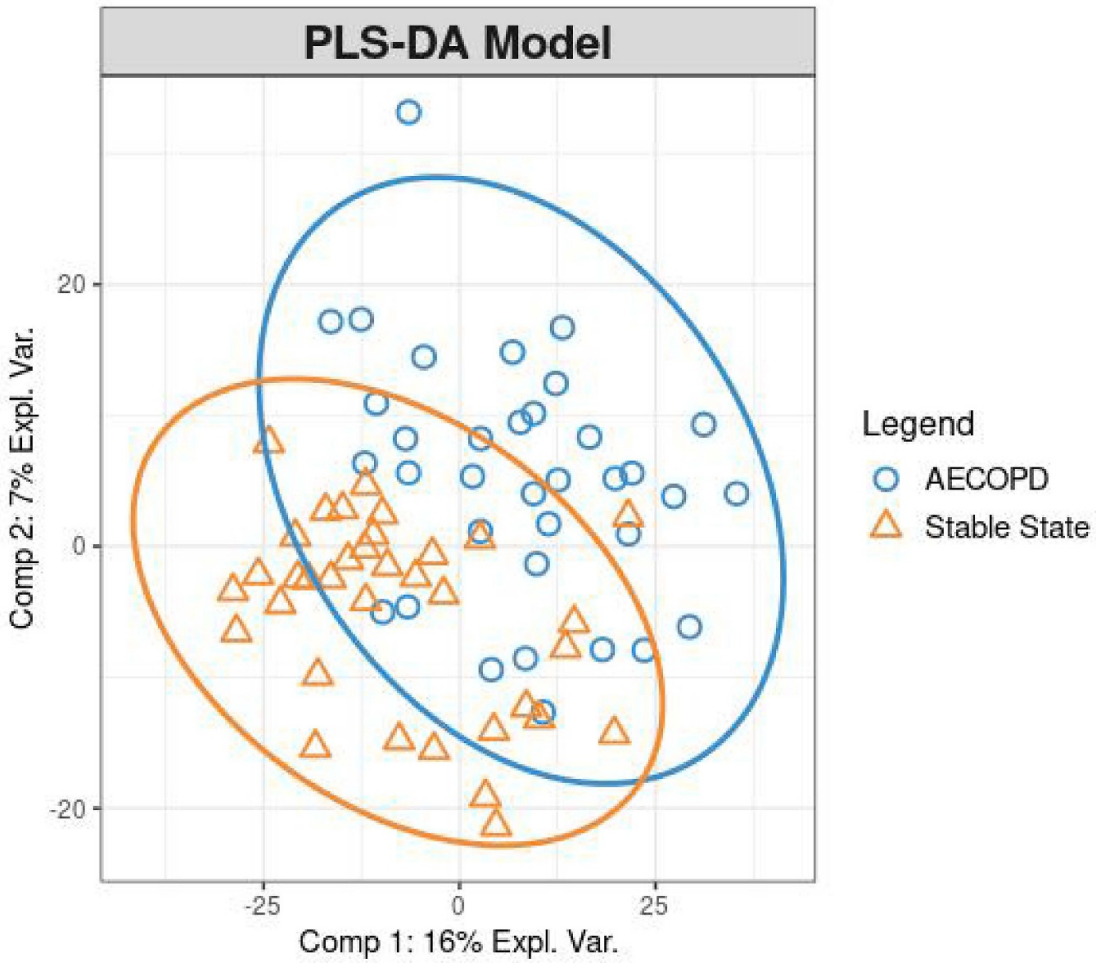

Figure 1. Partial least squares discriminant analysis (PLS-DA) plot discriminating AECOPD from stable state using the first two components. This PLS-DA plot illustrates the separation between the acute exacerbation of chronic obstructive pulmonary disease (AECOPD) and stable state groups. Each point represents a visit, color-coded based on their classification: blue circles for AECOPD and orange triangles for stable state. The *x*-axis (component 1) and *y*-axis (component 2) represent the first two components of the PLS-DA model, which capture the most significant variance between the groups. The model's explained variance by component 1 and component 2 are indicated on their respective axes. Clear clustering of orange triangles and blue circles indicates effective discrimination between AECOPD and stable state, demonstrating the utility of the selected biomarkers in distinguishing these clinical states.

The additional activation of the tryptophan metabolism in our study is in line with the literature on metabolic alterations during inflammatory processes. In response to an inflammatory stimulus, pro-inflammatory cytokines activate the degradation of tryptophan, mainly through the kynurenine pathway. Thus, decreased levels of tryptophan and increased levels of kynurenines are present during inflammatory processes [22]. Gulcev *et al* [23]. Previously showed decreased tryptophan levels in blood plasma of patients with AECOPD.

The linoleate metabolism was found to be an additional activated metabolic pathway. Linoleic acid, the most abundant PUFA in humans, is converted into lipid mediators such as pro-inflammatory leukotrienes and prostaglandins via the arachidonic acid pathway [24]. A study investigating induced sputum from patients with COPD and smoking controls showed reduced linoleic acid in patients with stable COPD [25]. We hypothesise that linoleate mainly feeds into the arachidonic acid pathway during inflammatory processes and therefore metabolites in other pathways of the linoleate metabolism are reduced.

Breath features of the linoleate, tyrosine, and tryptophan metabolism are in fact some of the most discriminating breath features out of our cluster enabling AECOPD prediction. This cluster with molecules from various metabolic pathways enables to predict AECOPD compared to a stable state with good sensitivity of 82.5% and excellent discriminative power (AUC = 0.86) in a non-invasive manner within minutes.

Our findings are highly promising that exhaled breath analysis could be a smart new technique to diagnose AECOPD in clinical settings. However, further studies are needed to investigate the discriminative power of exhaled breath in various differential

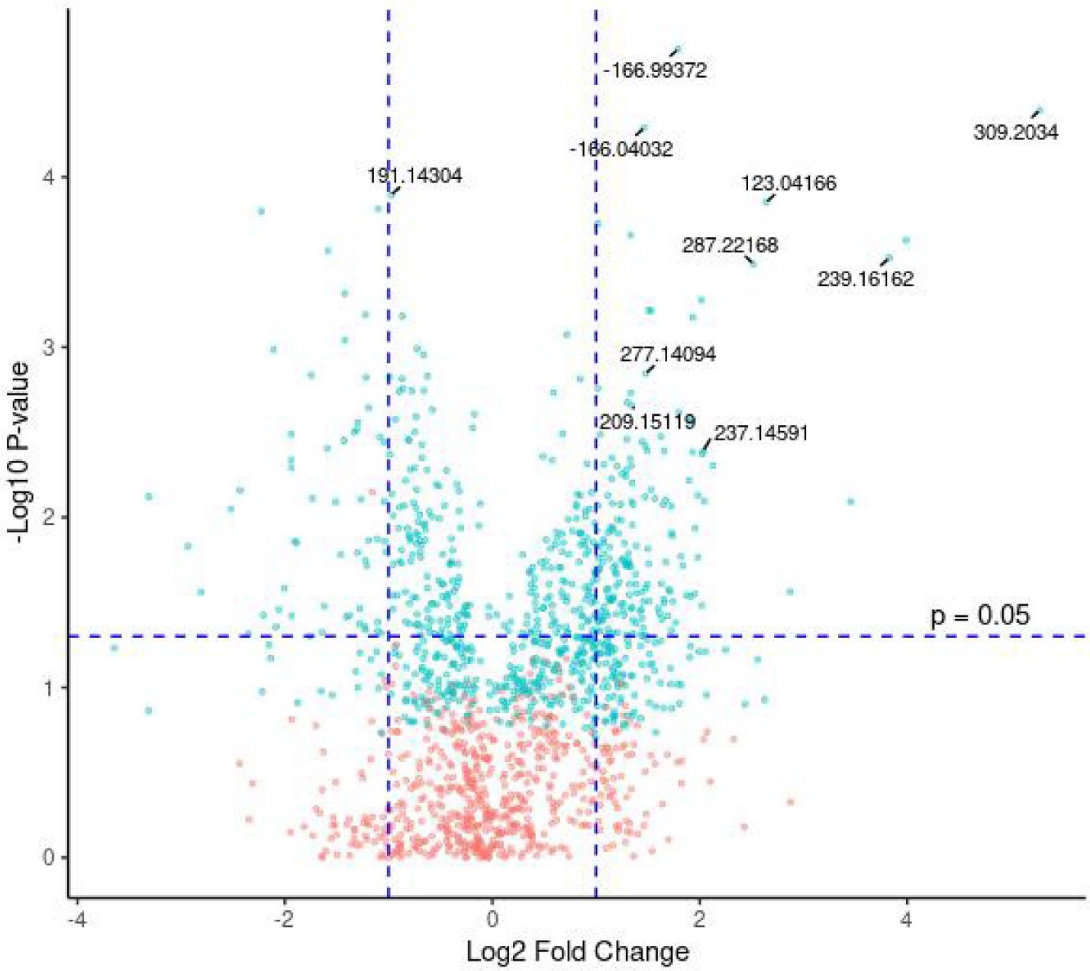

Figure 2. Volcano plot of breath features highlighting the most discriminative breath features from the PLS-DA model. This volcano plot visualises the differential expression of breath features between AECOPD and stable state (right/left relatively increased in AECOPD/ stable state). The *x*-axis represents the log2 fold change in expression levels between the two visits, while the *y*-axis represents the negative logarithm of the p-value, indicating statistical significance. Blue points represent features with a variable importance in projection (VIP) score < 1, indicating lower discriminative power in the PLS-DA model. Orange points represent features with a VIP score > 1, indicating higher discriminative power in the PLS-DA model. Features with higher log2 fold change and higher -log10 (p-value) are more significant in distinguishing between AECOPD and stable state. The most discriminative breath features from the PLS-DA model are labelled (m/z).

diagnoses of patients attending the emergency department with symptoms suggestive of AECOPD [26]. The implementation of our prediction model with a specificity of 77.1% demonstrates significant efficacy in correctly identifying patients in a stable state of COPD. This level of specificity could notably reduce the over- treatment of COPD patients who do not experience acute exacerbations, thereby preventing unnecessary use of steroids and/or antibiotics. In clinical settings, such as emergency departments, this approach may effectively identify patients with acute COPD exacerbations, enabling prompt and targeted treatment.

Furthermore, our results emphasise the need for continued research into exhaled breath analysis to identify additional biomarkers of AECOPD and thoroughly identify affected metabolic pathways during AECOPD. Further studies that reveal the chemical structure of specific breath metabolites involved in such 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 affected metabolic pathways have been postulated based on database matching of measured accurate masses (within 2ppm). The definitive identification of molecules remains a crucial task in the field of exhaled breath analysis, i.e. by the use of additional ultra-performance liquid chromatography—MS/MS analysis employing chemical standards.

Our study reveals substantial metabolic changes during acute exacerbations of COPD compared to stable state, suggesting alterations predominantly in the linoleate, tyrosine, and tryptophan pathways, which are all associated with inflammatory processes. The utilization of non-invasive and real-time

exhaled breath analysis could enable prediction of AECOPD and thus may be used to improve the precision and efficacy of AECOPD diagnosis in clinical practice.

Data availability statement

All data that support the findings of this study are included within the article (and any supplementary files).

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Guarantor: Malcolm Kohler takes responsibility for the content of the manuscript.

Conflict of interest

MK reports consulting fees from Novartis and GSK. MK is co- founder of DBI AG, a company that provides services in the field of breath analysis. SU reports personal fees from MSD, Janssen, Novartis, Orpha Swiss, Gebro SA. PS is co- founder and board member of DBI AG. FS is a part-time employee of DBI AG. None declared (SB, NAS, KF, AA, JH, DMB).

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