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Determination of Untargeted Metabolomic Profile in Breath Condensate Using LC-QTOF/MS and Investigation of Potential Lung Cancer-Specific Biomarkers

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INTRODUCTION: Lung cancer remains the leading cause of cancer-related mortality worldwide, primarily due to late diagnosis and the lack of sensitive, non-invasive biomarkers for early detection. Conventional diagnostic methods such as imaging and biopsy are either invasive or incapable of identifying disease at its earliest stages. Therefore, there is a growing demand for analytical strategies that enable non-invasive, rapid, and reliable diagnosis of lung cancer.[1] In recent years, exhaled breath condensate (EBC) has emerged as an attractive biological matrix in clinical metabolomics. EBC collection is completely non-invasive, painless, and repeatable, allowing real-time reflection of the metabolic status of the respiratory tract. It contains a variety of low-molecular-weight compounds, including volatile and non-volatile metabolites derived from airway and alveolar surfaces. Metabolomic profiling of EBC, particularly through high-resolution mass spectrometry, offers valuable insight into the biochemical alterations associated with lung cancer pathogenesis.[2] Metabolomics, as a systems biology approach, enables comprehensive analysis of metabolites that serve as the final products of cellular processes. This field provides a functional readout of the physiological state and disease progression, bridging the gap between genotype and phenotype. The untargeted metabolomics approach—unlike targeted methods that focus on predefined molecules—captures the widest possible range of metabolites, making it ideal for exploratory biomarker discovery.[3] The present study aimed to investigate potential lung cancer-specific biomarkers through untargeted metabolomic profiling of EBC samples using liquid chromatography coupled with quadrupole time-of-flight mass spectrometry (LC-QTOF/MS). Breath samples were collected from lung cancer patients and healthy controls, and data were analyzed using multivariate statistical approaches to identify significant metabolic differences between the two groups.

MATERIAL AND METHODS: EBC samples were collected in the morning after overnight fasting. Participants inhaled filtered air through their noses and exhaled through their mouths into a thermostat-controlled condenser system maintained at +4 °C for 15 minutes. Saliva contamination was prevented using a U-shaped glass trap. The collected condensate (approximately 2-2.5 mL) was transferred into Teflon tubes and stored at -80 °C until analysis. Metabolites were separated by LC-QTOF/MS, a high-resolution analytical platform suitable for untargeted metabolomics due to its excellent mass accuracy and sensitivity. Raw data were processed using Signpost MS software for peak alignment, normalization, and statistical comparison. Principal component analysis (PCA) and hierarchical clustering were

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applied to visualize group separation and identify metabolite patterns associated with lung cancer. The workflow of the method is shown in Figure 1.

RESULTS: The PCA score plot revealed a clear separation between lung cancer patients (Group A) and healthy controls (Group B) along the PC2 axis, accounting for 8.5% of total variance, while PC1 explained 21.7%. The partial overlap between groups indicated some biological heterogeneity within the cancer cohort, which is expected due to tumor stage and individual metabolic variability. Nevertheless, the distinct clustering pattern confirmed that EBC contains disease-specific metabolic information. A heatmap visualization demonstrated differential metabolite expression profiles between the two groups. Notably, several metabolites exhibited increased intensity in the cancer group, suggesting potential involvement in tumor metabolism. Subsequent statistical evaluation (P <0.05) identified six significant metabolites, among which L-alanine and creatine were highlighted as potential endogenous biomarker candidates. Both metabolites play essential roles in cellular energy metabolism and biosynthetic pathways. Elevated creatine levels in EBC may reflect altered energy demands and mitochondrial dysfunction characteristic of cancerous cells. Meanwhile, L-alanine accumulation is consistent with enhanced glycolytic flux ("Warburg effect"), a hallmark of cancer metabolism.[3] Additional compounds such as N-(2,4-dichlorophenyl) hydrazinecarboxamide and 4-dimethylaminobenzophenone were also detected, possibly representing exogenous exposures or metabolic byproducts of oxidative stress.These findings collectively indicate that EBC metabolomics provides a rich biochemical snapshot of lung pathophysiology. The untargeted LC-QTOF/MS approach offers both qualitative and quantitative insights into metabolite alterations, thereby establishing EBC as a promising diagnostic medium for early lung cancer screening.

CONCLUSION: The results of this study demonstrated that untargeted metabolomic profiling of EBC can successfully distinguish lung cancer patients from healthy individuals. Identified biomarkers such as L-alanine and creatine show potential for early disease detection and could serve as the foundation for developing non-invasive screening tools. Future studies should validate these findings in larger cohorts and explore longitudinal sampling to assess biomarker stability and diagnostic accuracy. The integration of high-resolution mass spectrometry with non-invasive sampling techniques represents a powerful direction in personalized oncology. Such approaches may complement existing diagnostic modalities and contribute to earlier detection, better prognosis, and improved patient outcomes.

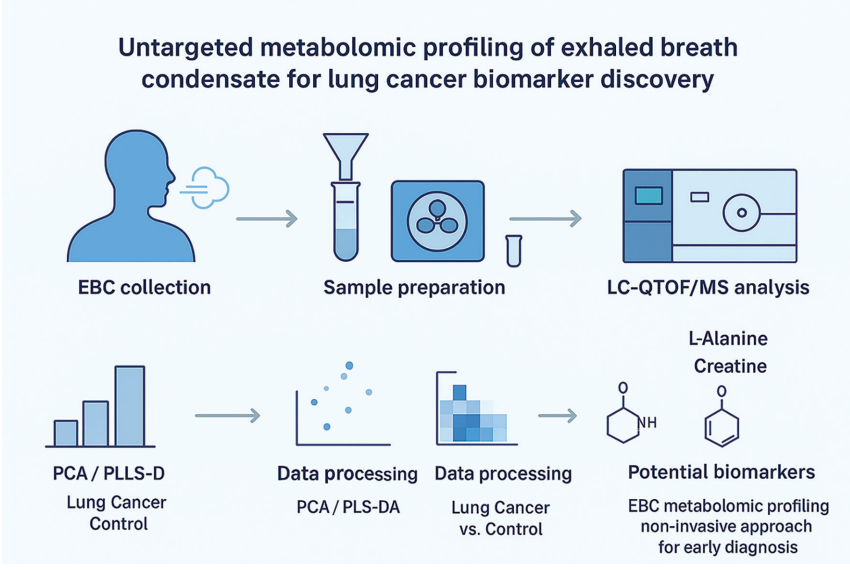

Figure 1: Graphical Abstract

KEYWORDS: Biomarker, quadrupole time-of-flight liquid chromatography/mass spectrometry, metabolomics, exhaled breath condensate, principal component analysis

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