Metabolic biomarkers in lung cancer screening and early diagnosis (Review)

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Abstract. Late diagnosis is one of the major contributing factors to the high mortality rate of lung cancer, which is now the leading cause of cancer-associated mortality worldwide. At present, low-dose CT (LDCT) screening in the high-risk population, in which lung cancer incidence is higher than that of the low-risk population is the predominant diagnostic strategy. Although this has efficiently reduced lung cancer mortality in large randomized trials, LDCT screening has high false-positive rates, resulting in excessive subsequent follow-up procedures and radiation exposure. Complementation of LDCT examination with biofluid-based biomarkers has been documented to increase efficacy, and this type of preliminary screening can potentially reduce potential radioactive damage to low-risk populations and the burden of hospital resources. Several molecular signatures based on components of the biofluid metabolome that can possibly discriminate patients with lung cancer from healthy individuals have been proposed over the past two decades. In the present review, advancements in currently available technologies in metabolomics were reviewed, with particular focus on their possible application in lung cancer screening and early detection.

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Key words: lung cancer, metabolic biomarkers, early diagnosis, blood, urine, exhaled breath

Contents

1. Introduction

Lung cancer is one of the most common malignancies and is the leading cause of cancer-associated mortality worldwide (1). The poor prognosis and high mortality rate of lung cancer are mainly due to late diagnosis (2). At present, only ~15% of newly diagnosed lung cancer cases are diagnosed in the early stages (stages I-II), which contributes to >60% probability of 5-year survival when effective treatment is available (3-5). However, >60% patients with lung cancer are first diagnosed already in the advanced stages (stage IV) or already with metastatic tumors, who typically only have 5-year survival rates of <5% (6).

In addition to the reduction in exposure to tobacco smoke, screening for the early detection of lung cancer has been considered to be a major strategy for decreasing the rate of lung cancer mortality (7). At present, low-dose CT (LDCT) screening in the high-risk population is the predominant tool used for detecting lung cancer in the early stages (2). The results of the US National Lung Screening Trial (ClinicalTrials.gov number, NCT00047385) found that compared with chest X-ray examination, LDCT screening was associated with a 20% reduction in lung cancer-specific mortality in a high-risk group of participants defined by their smoking status (8). In addition, other previous studies have also confirmed the validity of LDCT screening for the early detection of lung cancer to reduce mortality rate (9,10). However, potentially healthy individuals are also at risk of being subjected to expensive and

potentially harmful diagnostic procedures, such as positron emission tomography, transthoracic/bronchoscopic biopsy or even surgery, due to the considerably high false-positive rate of LDCT (nearly 96.4%) (8). Therefore, the combination of LDCT with additional biomarker-based tests has been proposed to be a more favorable strategy for improving the effectiveness of lung cancer screening programs whilst reducing the cost and harmfulness to otherwise healthy individuals (11). Such tests can either pre-select individuals from a high-risk population for LDCT examination or discriminate between benign and malignant pulmonary nodules detected by LDCT screening (12).

Several different components of blood, including specific serum/plasma proteins, autoantibodies, microRNA, cell-free DNA and circulating tumor cells, have all been proposed to be potential lung cancer biomarkers (13-15). However, they typically have low specificities and few were found for wider beneficial application in clinical practice, especially for the early detection of lung cancer. Instead, monitoring cancer-related metabolites is an emerging and promising approach for the detection and diagnosis of a number of malignant tumors, including colorectal, gastric, gynecological and lung cancer (16-20).

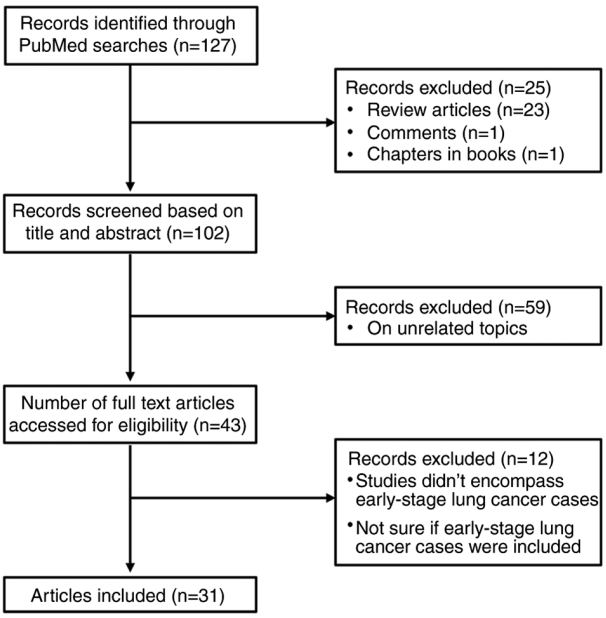

Figure 1. Flow chart depicting the identification and selection of articles.

2. Method

A search for articles published in English on PubMed (https://pubmed.ncbi.nlm.nih.gov/) was conducted regarding the use of metabolic biomarkers in lung cancer screening and early diagnosis that were published between Jan 1, 2002 and Aug 1, 2022. The search terms used were 'lung cancer', 'metabolites', and 'early detection'. A total of 127 unique articles were identified and examined. Of these 127 articles, 25 were excluded due to being inappropriate article types, such as chapters in books, comments or review articles. Among the remaining 102 articles, 59 articles were removed due to being on unrelated topics, such as those not on lung cancer, not on cancer detection, not on metabolomics or not on biofluids (blood, urine and exhaled breath). Following evaluation of the full text of the remaining 43 articles, 12 were rejected, as these studies did not encompass early-stage lung cancer cases or it was unclear if early-stage lung cancer cases were included in the studies. Finally, the present review included 31 articles for analysis, as shown in the flow chart in Fig. 1.

3. Metabolomics: A new source of cancer biomarkers

Metabolomics, also known as metabolic profiling, uses quantitative and qualitative analyses to determine key metabolism-associated molecules of different molecular masses (21,22). It reveals information into specific states of cancer that are otherwise not apparent (22). Previously, the assessment of metabolic changes is limited to measuring the levels of individual hormones and metabolites using imaging modalities and standard clinical laboratory tests (23). By contrast, metabolomics involves the measurement of vast numbers of metabolites systematically, including carbohydrates, nucleotides, carboxylic acids, amino acids and lipids in blood, urine, or other body fluids (24). Metabolomics has emerged to be a potentially powerful approach for identifying cancer biomarkers and drivers of tumorigenesis (25). In addition, compared with other 'omes', such as genome, transcriptome, and the proteome, the metabolome reflects the real-time status of a particular phenotype to reveal what exactly has happened in the organisms exactly, providing *bona fide* biomarkers for disease surveillance (Fig. 2).

4. Metabolomics techniques and technologies

The main methodologies involved in metabolomics have been based on nuclear magnetic resonance (NMR) spectroscopy and mass spectrometry (MS) techniques, coupled with either gas chromatography (GS) or liquid chromatography (LC), each with their specific advantages and limitations (Table I). NMR is highly selective and non-destructive, rendering it recognized to be the gold standard for elucidating the structure of metabolites. However, the sensitivity of NMR is relatively low, only being able to detect metabolites with concentrations $>10^{-5}$ M (26). By contrast, MS has higher sensitivity and selectivity (26). Modern MS provides highly specific chemical information, such as accurate molecular mass, isotope distribution patterns for element formulation determination, and characteristic fragment-ion information directly related to the chemical structure of metabolites (27). In addition, the high sensitivity of MS allows for the detection and measurement of a large number of primary metabolites (the initial end products created by a live organism as a result of growth) and secondary metabolites (aid in the performance of various biological tasks that are not engaged in the growth and maintenance of cellular activity) at picomolar to femtomolar levels. As one of the major tools for the collection of 'omic' information, MS techniques use big data for processing and interpretation by machine learning (28,29). These unique advantages make MS an essential tool for metabolomics analysis (30). Over the past decade, various comprehensive reviews have already discussed how NMR and MS work, and how each can be used for metabolomics (31-33). Despite their own advantages and disadvantages, several studies have shown how they can

Table I. Comparison between NMR and MS.

Properties	NMR	MS
Sensitivity	Low	High, at picomolar and femtomolar levels
Selectivity	Generally used for non-selective analysis	Generally used for selective and non-selective (targeted and untargeted) analysis
Sample measurement	All metabolites with NMR concentration levels can be detected in one assay	Different chromatographic techniques are required for different types of metabolites
Sample recovery	i) Non-destructive; and ii) samples can be recovered and stored for a long period; iii) samples can be analyzed multiple times	i) Destructive; and ii) samples can't be recovered
Reproducibility	High	Moderate
Sample preparation	Small sample volumes	i) High quality of sample preparation; and ii) different columns and optimized ionization conditions are required
Number of metabolites detected	40-200, depending on spectral resolution	≥500

NMR, nuclear magnetic resonance; MS, mass spectrometry.

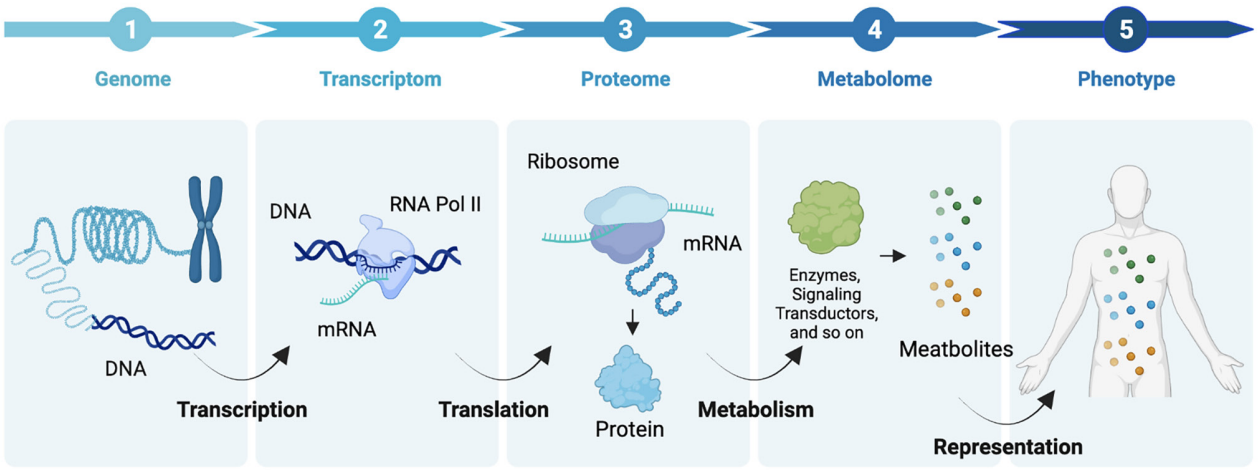

Figure 2. Relationship between *-omes* with systems biology. Cancer is caused by changes at the genomic level which results in altered RNA transcription, protein expression and function. The metabolome reflects the real-time status of phenotype and reveals what exactly has happened in the organisms.

be used to complement each other (31-35). Indeed, the use of multiple technologies greatly broadens the level of metabolite coverage and the types of samples that can be studied (36).

5. Metabolomics for the early detection of lung cancer

Over the past two decades, a number of metabolomics studies have been performed based on NMR and/or MS techniques to generate metabolite profiles that can discriminate patients with lung cancer from healthy individuals using different types of biological samples. The present review summarizes the studies that include early-stage lung cancer cases to evaluate the viability of using metabolomics for the early detection of lung cancer.

Blood metabolomics. Blood samples, including plasma and serum, are the most widely studied biological fluids for lung cancer research. It can be used for characterizing metabolic markers, using both targeted (measuring a specific set or family of compounds only) and untargeted (global profiling) approaches (Table II) (37-54).

Previous studies have described alterations in the levels of amino acids, especially in alanine, glutamine, histidine, leucine, isoleucine, lysine and serine, in the serum or plasma of patients with lung cancer (37-42,44). These alterations may be associated with the increase in amino acid demand caused by the proliferation of the tumor cells, highlighting the important role of amino acid metabolic pathways in lung cancer progression.

Lactic acid is another commonly altered metabolite in patients with lung cancer (37,39). Previous studies on lactate metabolism in patients with cancer have suggested that changes in the level of this metabolite are due to the increased glucose uptake and lactate production by the tumor in the absence of oxygen (55,56). In support of this, in various tumor

Table II. List of metabolic studies of blood and urine applied to lung cancer.

A, Serum and plasma

First author, year	Research subjects	Analytical techniques	Major findings	(Refs.)
Rocha *et al*, 2011	85 LC (69 eLC) and 78 CTR	NMR	Compared with CTR samples, LC samples had significantly: i) Higher levels of LDL, VLDL, lactic acid and pyruvic acid; and ii) lower levels of HDL, glucose, citrate, formate, acetate, amino acids (such as alanine, glutamine and histidine) and methanol	(37)
Deja *et al*, 2014	77 LC (35 eLC) and 22 COPD	NMR	Compared with COPD samples, LC samples had significantly: i) Higher levels of N-acetylated glycoproteins, leucine, lysine, mannose, choline and lipids; and ii) lower levels of acetate, citrate and methanol	(38)
Klupczynska *et al*, 2016	90 LC (70 eLC) and 63 CTR	Liquid chromatography -MS	A new set of six amino acids (aspartic acid, β-alanine, histidine, asparagine, phenylalanine and serine) ensured higher accuracy to distinguish LC from CTR (from 90.3 to 77.1% depending on the histological type)	(44)
Puchades-Carrasco *et al*, 2016	142 LC (72 eLC) and 87 CTR	NMR	Compared with CTR samples, LC samples had significantly: i) Higher levels of leucine/isoleucine, acetate, N-acetyl-cysteine, glutamate, methanol, glycerol, creatine and lactic acid; and ii) lower levels of HDL, LDL, VLDL, adipic acid, lipids, glutamine, Choline–$N(CH_3)_3$, threonine and histidine	(39)
Louis *et al*, 2016	331 LC (93 eLC) and 315 CTR	NMR	Compared with CTR samples, LC samples had significantly: i) Higher levels of glucose, N-acetylated glycoproteins, b-hydroxybutyrate, leucine, lysine, tyrosine, threonine, glutamine, valine and aspartate; and ii) lower levels of alanine, lactate, sphingomyelin and phosphatidylcholine (and other cholinated phospholipids), citrate and other phospholipids	(45)
Klupczynska *et al*, 2017	50 eLC and 25 CTR	UHPLC-Q-Orbitrap-HRMS	In total, 36 metabolites were significantly altered between LC and CTR samples, including carnitine, acyl-carnitines, malic acid, pyroglutamic acid, histidine and histamine. A signature consisting of 12 of these identified metabolites allowed the building of a cancer classifier characterized by a receiver operating characteristic AUC value of 0.836	(41)
Ros-Mazurczyk *et al*, 2017	31 LC (25 eLC) and 92 CTR	GC-MS	Compared with CTR samples, LC samples had significantly: i) Higher levels of benzaldehyde; and ii) lower levels of 17 metabolites, including amino acids, carboxylic acids and alcohols	(40)
Chen *et al*, 2015	30 LC (22 eLC) and 30 CTR	GC-MS and liquid chromatography -MS	Compared with CTR samples, LC samples had significantly: i) Higher levels of phosphorylcholine, glycerophospho-N-arachidonoyl, ethanolamine, γ-linolenic acid, α-hydroxyisobutyric acid and 9,12-octadecadienoic acid; and ii) lower levels of prasterone sulphate, sphingosine, serine and 2,3,4-trihydroxybutyric acids	(42)
Mazzone *et al*, 2016	94 LC (57 eLC) and 190 CTR	GC-MS and liquid chromatography -MS	79 metabolites significantly increased and 70 metabolites decreased in the LC group. In total, the ratios of 9,723 metabolites differed significantly between the LC and CTR groups	(46)

Table II. Continued.

A, Serum and plasma

First author, year	Research subjects	Analytical techniques	Major findings	(Refs.)
Yu *et al*, 2017	199 eLC and 147 CTR	ESI-MS	LC samples had significantly increased levels of LPE(18:1) and ePE(40:4) but decreased levels of C(18:2)CE and SM(22:0) compared with those in CTR samples	(47)
Ros-Mazurczyk *et al*, 2017	100 eLC and 300 CTR	MALDI-MS and liquid chromatography -MS	PC, diacylophospholipids and SMs were frequently upregulated, whilst LPC 18:2, LPC 18:1 and LPC 18:0 were significantly downregulated in LC samples	(43)
Xiang *et al*, 2018	99 LC (33 eLC) and 112 CTR	Liquid chromatography -MS	In total, 28 endogenous metabolites were present at significantly different levels in patients with LC compared with those in CTR group. Cortisol, cortisone and 4-methoxyphenylacetic acid had high sensitivity and specificity values (AUC=0.955) as biomarkers for discriminating between LC and CTR	(48)
Klupczynska *et al*, 2019	20 eLC and 20 CTR	Liquid chromatography -MS	Compared with CTR samples, LC samples had significantly: i) Higher levels of PC (PC 42:4, 42:1, 44:3 and 40:2) and LPC (LPC 26:0 and 26:1); and ii) lower levels of PC 34:4. A biomarker panel of the seven aforementioned metabolites was able to distinguish eLC from CTR with a sensitivity of 70-90% and a specificity of 90-93%	(49)
Zhang *et al*, 2020	156 LC (130 eLC) and 60 CTR	Liquid chromatography -MS	β-hydroxybutyric acid, LPC 20:3, PC acyl ester C40:6, citric acid and fumaric acid were significantly different between CTR and eLC samples	(50)
Huang *et al*, 2020	200 eLC and 200 CTR	LDI-MS	Compared with CTR samples, LC samples had significantly: i) Higher levels of cystenie, histamine, fatty acid, uracil and uric acid; and ii) Lower levels of hydroxypicolinic acid and indoleacrylic acid. A biomarker panel of these seven metabolites was able to distinguish eLC from CTR with a sensitivity of 70-90% and a specificity of 90-93%	(51)
Derveaux *et al*, 2021	114 LC (48 eLC) and 118 CTR	NMR	In total, 62 metabolites, including lactate, valine, alanine, maleic acid and phenylalanine, were significantly different between LC and CTR samples	(52)
Qi *et al*, 2021	98 LC (52 eLC) and 75 CTR	Liquid chromatography -MS	In total, five metabolites (palmitic acid, heptadecanoic acid, 4-oxoproline, tridecanoic acid and ornithine) had the potential for early lung cancer screening. The discrimination accuracy and AUC score reached as high as 0.829 and 0.869	(53)
Wang *et al*, 2022	171 eLC and 140 CTR	Liquid chromatography -MS	In total, nine lipids (LPC 16:0, 18:0 and 20:4; PC 16:0-18:1, 16:0-18:2, 18:0-18:1, 18:0-18:2; and 16:0-22:6 and triglycerides 16:0-18:1) were identified as the features most important for early-stage cancer detection	(54)

Table II. Continued.

B, Urine

First author, year	Research subjects	Analytical techniques	Major findings	(Refs.)
Hanai *et al*, 2012	20 LC (11 eLC) and 20 CTR	GC-MS	Tetrahydrofuran, 2-chloroethanol, 2-pentanone, 2-methylpyrazine, cyclohexanone, 2-ethyl-1-hexanol, 2-phenyl-2-propanol, isophorone and 2,6-diisopropylphenol were significantly upregulated in LC samples	(62)
Mathe *et al*, 2014	469 LC (211 eLC) and 536 CTR	UPLC-ESI-QTOF-MS	Levels of creatine riboside, nacetylneuraminic acid, cortisol sulfate and an unidentified glucuronidated compound referred to as '561+' were significantly elevated in the LC group	(63)
Haznadar *et al*, 2016	178 LC (28 eLC) and 351 CTR	Liquid chromatography -MS	Creatine riboside was associated with lung cancer risk in the overall case-control set, whilst creatine riboside and nacetylneuraminic acid were associated with lung cancer risk in a European-American cohort	(64)
Funai *et al*, 2020	46 LC (23 eLC) and 185 CTR	Liquid chromatography -MS and NMR	O-aminohippuric acid was significantly upregulated in LC samples compared with that in CTR samples	(65)

LC, lung cancer; eLC, early lung cancer; CTR, control; COPD, chronic obstructive pulmonary disease; NMR, nuclear magnetic resonance; MS, mass spectrometry; GC-MS, gas chromatography-MS; UHPLC-Q-Orbitrap-HRMS, ultra-high-performance liquid chromatography-quadrupole-Orbitrap-high-resolution MS; ESI, electrospray ionization; MALDI, Matrix-Assisted Laser Desorption Ionization; LDI, laser desorption/ionization; UPLC-ESI-QTOF, ultraperformance liquid chromatography-electrospray-ionization-quadrupole time-of-flight; AUC, area under the curve; LDL, low density lipoprotein; VLDL, very low-density lipoprotein; HDL, high-density lipoprotein; PC, phosphatidylcholines; LPC, lysophosphatidylcholine; SM, sphingomyelin.

cells such as lung carcinoma, renal carcinoma and gastric carcinoma cells, glucose is preferentially catabolized through fermentation into lactate even when oxygen is not limiting, in a phenomenon known as the Warburg effect (55,57).

Phospholipids are important constituents of the cell membrane (58). Phospholipid metabolic pathways are regularly found to be dysregulated in lung cancer, yielding distinct signatures (58). Several studies have revealed perturbed levels of phospholipids in the blood of patients with lung cancer (42,43,46,47,54). Yu *et al* (47) previously performed a study based on electrospray ionization-MS and found that lung cancer samples had significantly increased levels of lysophosphatidylethanolamine(18:1) and phosphatidylethanolamine(40:4), and decreased levels of cholesterol ester(18:2) and sphingomyelin(22:0) compared with those in healthy controls. The cancer classifier built using these four phospholipids was characterized by Area under the ROC Curve (AUC) values to be 82.3 and 80.8% in the training and validation set, respectively (47). Another previous study based on LC-MS encompassed 100 patients with early lung cancer and a matched group of 300 healthy individuals regarding sex, age and smoking exposure. Upregulation of phosphatidylcholines (PC), diacylophospholipids and SMs coupled with the downregulation of lysophosphatidylcholines (LPCs), such as LPC 18:2, LPC 18:1 and LPC 18:0 distinguished lung cancer cases from controls. An effective cancer classifier composed of seven components was built with an AUC of 0.88 (43). Recently, Wang *et al* (54) performed single-cell RNA sequencing of different early-stage lung cancers and found that global aberration of lipid metabolism in various cell types, including T cells, B cells, fibroblasts, endothelial cells and epithelial cells, with glycerophospholipid metabolism as the most altered (with the smallest P-value) lipid metabolism-related pathway. Untargeted lipidomics was performed in an exploratory cohort of 311 participants with nine phospholipids (LPC 16:0, 18:0 and 20:4; PC 16:0-18:1, 16:0-18:2, 18:0-18:1, 18:0-18:2 and 16:0-22:6; and triglycerides 16:0-18:1-18:1) being identified to be the features most beneficial for early lung cancer detection. Using these nine features, a LC-MS-based targeted assay achieved 100.0% specificity for early lung cancer detection on an independent validation cohort (54). In a hospital-based lung cancer screening cohort of 1,036 participants examined by LDCT and a prospective clinical cohort containing 109 participants, the assay reached ≥90.0% sensitivity and 92.0% specificity for the discrimination of patients with lung cancer compared with healthy controls (54).

Urine metabolomics. Compared with other biospecimens, urine can be obtained from larger cohorts non-invasively and is therefore accepted more easily by the public. Similar

Table III. Metabolic studies of exhaled breath applied to LC.

First author, year	Research subjects	Analytical techniques	Major Findings	Sensitivity, %	Specificity, %	(Refs.)
Phillips *et al*, 1999	87 LC (16 eLC) and 91 CTR	GC-MS	A predictive model employing nine VOCs (butane, tridecane, tridecane, octane, hexane, heptane, hexane, pentane and decane) exhibited sufficient sensitivity and specificity to be considered as a screen for LC in a high-risk population	85.1	80.5	(70)
Poli *et al*, 2005	36 eLC, 25 COPD and 50 CTR	GC-MS	The combination of the 13 VOCs allowed the correct classification of the cases into groups. Together with conventional diagnostic approaches, VOC analysis could be used as a complementary test for the early diagnosis of LC	72.2	93.6	(71)
Phillips *et al*, 2007	193 LC (120 eLC) and 211 CTR	GC-MS	Mean typicality scores employing a 16-VOC model were significantly higher in patients with LC compared with those in the control group. The predictive model achieved near-maximal performance with six breath VOC	84.6	80.	(72)
Fu *et al*, 2014	97 LC (50 eLC), 32 BPN and 88 CTR	FT-ICR-MS	The concentrations of 2-butanone, 2-hydroxyacetaldehyde, 3-hydroxy-2-butanone and 4-hydroxyhexenal in the exhaled breath of patients with LC were significantly higher compared with that in the BPN and CTR samples	89.8	81.3	(73)
Li *et al*, 2015	85 LC (44 eLC), 34 BPN and 85 CTR	FT-ICR-MS	A model based on elevated levels of the six carbonyl VOCs (2-butanone, 2-hydroxyacetaldehyde, 3-hydroxy-2-butanone, 4-hydroxyhexenal, acrolein and malondialdehyde) effectively discriminates patients with LC from healthy controls in addition to patients with BPN	96	84	(74)
Sakumura *et al*, 2017	107 LC (70 eLC) and 29 CTR	GC-MS	In total, 68 VOCs were detected as LC markers and a combination of five VOCs (CHN, methanol, CH_3CN, isoprene and 1-propanol) was sufficient for 89.0% screening accuracy	95	89	(75)
Rudnicka *et al*, 2019	108 LC (17 eLC) and 121 CTR	GC-MS	In total, 88 VOCs were identified in the exhaled breath. The statistical analysis revealed seven analytes (acetone, methyl acetate, isoprene, methyl vinyl ketone, cyclohexane, 2-methylheptane and cyclohexanone) to have the highest discriminatory power	Validation group, 80; test group, 86.4	Validation group, 91.2; test group, 86.4	(76)

Table III. Continued.

First author, year	Research subjects	Analytical techniques	Major Findings	Sensitivity, %	Specificity, %	(Refs.)
Chen *et al*, 2021	160 LC (74 eLC), 70 BPN and 122 CTR	GC-MS	In total, 20 VOCs discriminated LC from CTR (AUC=0.987); 19 VOCs discriminated LC from BPN (AUC=0.809)	NR	NR	(77)
Tsou *et al*, 2021	148 LC (8 eLC) and 168 CTR	SIFT-MS	In total, 116 VOCs were analyzed in the exhaled breath samples and a quantitative VOCs databank integrated with the application of an XGBoost classifier (a machine learning method) provided a persuasive platform for lung cancer prediction	96	88	(78)

LC, lung cancer; eLC, early lung cancer; CTR, control; COPD, chronic obstructive pulmonary disease; BPN, benign pulmonary nodules; GC-MS, gas chromatography-mass spectrometry; NR, not reported; FT-ICR-MS, Fourier transform-ion cyclotron resonance mass spectroscopy; SIFT-MS, selected ion flow tube mass spectrometry; VOC, volatile organic compound; AUC, area under the curve.

to blood samples, urine also contains useful metabolic information for detecting the occurrence of lung cancer (59). The composition of urine is naturally less complex in comparison with that of plasma or serum, making it more popular for metabolomics analysis (59). In addition, once the blood is filtered by the glomerulus, certain components like metabolites can be concentrated in the urine, making their detection easier compared with that of other types of biological fluids. The first study to measure potential urinary metabolomic cancer biomarkers based on NMR and MS was published in 2006 and the concentration of nucleosides was found to be significantly increased in patients with breast cancer when compared with the normal controls (60). Carrola *et al* (61) first showed the valuable potential of using NMR-based metabolomics for finding putative biomarkers of lung cancer in the urine in 2011. Previous studies have since analyzed urinary metabolites employing either NMR or MS for early detection of lung cancer (Table II) (62-65).

The majority of urine metabolomic studies into patients with lung cancer found alterations in creatine and creatinine, making creatine and creatinine potentially valuable biomarkers for early lung cancer detection (61,63). To further assess whether creatine was elevated in the urine samples of subjects prior to lung cancer diagnosis, a detailed prospective study based on LC-MS was previously conducted (64). Urine samples from 178 patients with lung cancer and 351 volunteers were collected and examined, where it was revealed that elevated creatine levels were associated with lung cancer risk in both European- and African-Americans (64). Consistently, creatine and creatinine have been shown to be upregulated in other biofluids, such as serum and saliva, in patients with lung cancer (39,66). In the human body, creatine is synthesized from methionine, glycine, and arginine, which is then converted into creatinine (67). Therefore, the increase in creatine and creatinine levels may be associated with upregulated amino acid metabolism. Nevertheless, the promising results of urinary creatine and creatinine in early lung cancer detection remain to be validated by independent studies based on material collected real-time during lung cancer screening.

Exhaled breath metabolomics. Normal metabolism produces a variety of volatile organic compounds (VOCs), which can be expelled through respiration (68). Therefore, exhaled breath has also been explored as a potential source of cancer biomarkers. It was first shown that VOCs in the exhaled breath could be used to differentiate patients with lung cancer from healthy individuals in 1999 (69). Since then, accumulating evidence has been supporting the utility of VOC detections in the exhaled breath for the early detection of lung cancer, most yielding high degrees of sensitivity and specificity (Table III) (70-78).

Lung cancer causes oxidative stress and induces oxidase enzymes in tumor tissues, which in turn produce higher concentrations of specific VOCs, especially carbonyl VOCs in the exhaled breath. Carbonyl VOCs are produced by biochemical pathways, such as the respiratory chain and oxidative phosphorylation pathway, as intermediates, some of which can yield unique information into specific pathways, such as lipid oxidation induced by free radicals (79,80).

Consequently, several studies have focused on the identification of carbonyl VOCs as markers of lung cancer in the exhaled breath (73,74,81). Fu *et al* (73) previously found that the concentrations of 2-butanone, 2-hydroxyacetaldehyde, 3-hydroxy-2-butanone and 4-hydroxyhexenal (4-HHE) in the exhaled breath of patients with lung cancer were significantly higher compared to those in the exhaled breath of healthy controls and patients with benign pulmonary nodules (BPN). This was found using Fourier transform-ion cyclotron resonance MS (73). Bousamra *et al* (81) then showed that the sensitivity and specificity of breath analysis was associated with the number of the elevated VOCs. Among patients with lung cancer, three or four elevated cancer markers (2-butanone,

2-hydroxyacetaldehyde, 3-hydroxy-2-butanone and 4-HHE) produced a specificity of 95% to discriminate patients with lung cancer from healthy controls. Furthermore, an enhanced model based on the elevated levels of the six carbonyl VOCs, including the four markers identified in Fu's work (73), plus acrolein and malondialdehyde, was found to effectively discriminate patients with lung cancer from healthy individuals in addition to patients with BPN (73). The sensitivity in each case was ≥96%, with specificity ranging from 64% for BPN to 86% for smokers and 100% for non-smokers and for the three groups combined 84% (74).

Other potential metabolic biomarkers that can be used for the early detection of lung cancer were also obtained in previous studies. Typical examples are isoprene, methanol and acetone (71,75,76). Despite these promising advances, the lack of normalization and standardization has led to significant variations in the VOC profiles and/or concentrations among the different studies and no commercial products have been used in clinical practice due to the lack of uniform sampling standards and sample storage methods.

6. Conclusions

The metabolome is the most representative of the molecular phenotype of an organism, where the concentrations of metabolites directly reflect the current biochemical activity of the organism. Therefore, metabolomics is considered a suitable approach for increasing the efficacy of detecting early-stage lung cancer. However, such applications require a deeper understanding into how these measurements relate are associated with human physiology and cancer pathology. It remains to be elucidated which metabolites can be measured in biofluids which are readily accessible to accurately reflect cancer status. Although progress has been made, it remains unclear to what extent the metabolites in biofluids can reveal about the metabolic activity of the tumor. Additional metabolomics experiments in fluids associating these measurements to the physiology of cancer would be a promising future direction.

In addition, global metabolic alterations in biofluids do not allow for the differentiation of cancer from other diseases with systemic metabolic alterations such as hypercholesteremia and phenylketonuria. The issue of such confounding effects in metabolomic analysis will be minimized if the tumor tissues are tested appropriately using NMR and MS. Therefore, it would be of benefit to identify differentiating metabolites in tissues through untargeted metabolomics before testing them in the biofluids through targeted metabolomics. Several studies have recently highlighted the role of extracellular vesicles (EVs) and their cargo (protein and RNAs) in lung cancer diagnosis (82-85), proposing EVs to be another potential source of cancer biomarkers. Therefore, combined metabolomics approaches for EVs phenotyping would provide vital insights into the characteristics of EVs in cancer and potentially identify novel strategies for the early detection of lung cancer.

One of the challenges with metabolomics is the vast number and chemical complexity of metabolites that exist, such that no current metabolomics approach can cover these complexities comprehensively. This leads to inaccuracy in the early detection of lung cancer. The present review proposes that currently, the optimal metabolomics method for research would be combination with other 'omics' approaches to comprehensively elucidate the changes in metabolites in lung cancer whilst also to improve the accuracy of lung cancer screening.

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Availability of data and materials

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Authors' contributions

YJX and XSD were involved in conceptualization, writing, and reviewing. CQ, FW and JL were involved in writing, and reviewing. YWY, LZ, FWT, WQC and WC were involved in reviewing and editing. JH and NL were involved in study concept and design, draft manuscript preparation and analysis and interpretation. All authors reviewed the paper and approved the final version of the manuscript. Data authentication is not applicable.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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