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Review Article

Early diagnosis and screening in lung cancer

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Abstract: Lung cancer is the third most diagnosed cancer, but the first cause of cancer-related deaths worldwide. This rather high death rate is due mainly to the fact that most patients are diagnosed with advanced-stage cancer, for which the conventional treatment does not work. The most used screening method for lung cancer is a low-dose CT scan, but it is recommended for specific age populations and it also started different debates on its advantages for lung cancer diagnosis. Over the year, several new techniques have been developed that are less invasive, have lower side effect, and can be implemented at all types of populations. This article aimed to present the advantages and disadvantages of using several methods for lung cancer diagnosis, including analysis of volatile organic compounds, exhaled breath condensate analysis and specific genomic approaches.

Keywords: Lung cancer, early diagnosis, volatile organic compounds, exhaled breath condensate, genomic approaches

Introduction

Lung cancer is the third most diagnosed cancer, but the most prevalent cause of cancer-related deaths worldwide [1]. This rather high death rate is due mainly to the fact that most patients are diagnosed with advanced-stage cancer, for which the conventional treatment does not work [2]. In order to overcome this problem, the US changed in 2013 the guidelines for lung cancer screening and recommended low-dose CT (LDCT) scan for adults between 55 and 80 years that smoke 30 packs yearly or have quit smoking less than 15 years [3]. Different trials tried to identify different screening methods for lung cancer diagnosis, and it was observed that chest radiography or sputum sample are less efficient [4] than LDCT [5]. The choosing of screening methods has aroused different debates regarding the pros and cons of CT scan and how it can be implemented with a larger population range. In this way, Hofmann et al. presented in their analysis on LDCT that its implementation is costly but it presents a better alternative that standard CT scans, obtaining a risk reduction of lung cancer between 0.76-4.7 percent [6]. Previous data suggested that in order to improve the early diagnosis of lung cancer, better selection of target population, based on age and smoking habit are not enough [7]. Therefore, several methods based on the analysis of volatile organic compounds (VOCs), exhaled breath condensate analysis (EBC), or specific genomic approaches have been developed to improve the early diagnosis of lung cancer. The term EBC was firstly described in 2001 by an international organization formed to evaluate its use and define standardization factors for collection, storage, measurements, and contaminant. It was demonstrated that EBC collection is a non-invasive technique that can use portable devices, which makes it easy to implement in any situation and can be used in different epidemiologic studies. Its main drawback at that moment was the small num-

Table 1. Methods used for VOCs analysis

Method	Detection limit	Sensitivity	Sensibility	Type of detection	Ref
SIFT-MS	ppb	High	High	Real-time	[52]
PTR-MS	ppt	High	Medium high	Real time	[52]
IMS	ppb	Medium	Medium	Real time	[53]
Sensor array	ppb	Medium	Medium	Reference to a database	[28]
GC-MS	ppt-ppb	Very-High	Very-high	Pre-concentration	[28]
LAS	ppb	High	High	Real-time	[54]

ppb - parts per billion in volume, pptb - parts per trillion in volume, SIFT-MS - selected ion flow tube mass spectrometry, PTR-MS - proton transfer reaction mass spectrometry, IMS - ion mobility spectrometry, GC-MS - gas chromatografy-mass spectrometry, LAS - laser absorption spectroscopy.

ber of studies on biomarkers identified in EBC, which the task force determines to be not yet ready to use in the clinic [8], but the research could overcome this thing in this specific area. In 2017, the same task force made another analysis of EBC and observed that now there are a large number of studies in this area in different diseases, but still there is not a standardized way to evaluate biomarkers in EBC, mainly because there are no validated reference values for these biomarkers [9]. There are reference values only for some biomarkers like H_2O_2, 8-isoprostane, adenosine, pH, and leukotrienes [8, 10, 11]. The VOC terminology was introduced several years ago and it comprised all the volatile organic compounds that can be found in the human body. In a previous analysis, B de Lacy Costello et al. [12]. described the VOCs that can be found in the healthy human body and how they can be metabolized, also establishing a database of VOCs from healthy humans that can be used as reference when compared to disease VOCs [12]. The VOCs may be analyzed either by standard breathomics methods [13-16] or even by high throughput techniques that are included in areas of genomics, metabolomics or proteomics [17-20].

Consequently, in this article, we present up-to-date information about the advantages and disadvantages of using analysis of volatile organic compounds (VOCs), exhaled breath condensate analysis and specific genomic approaches to improve the diagnoses of lung cancer.

Analysis of VOCs in exhaled breath

Previous data have suggested that some volatile organic compounds (VOCs) contained in breath could be useful for lung cancer detection [21-23]. VOCs are organic compounds with a high vapor pressure at room temperature. They can show a distinct pattern in the pathological state, and this pattern is affected by a modification that appears in different cellular processes. After production, VOCs are excreted in blood from where they get in the lungs and exhaled [24]. These compounds have shown a great interest in lung cancer, mainly because patients with lung cancer are diagnosed in advanced stages [25, 26] but also because lung cancer diagnosis is challenging [27]. Since the detection of VOCs in breath is in the range of parts per billion (ppb), several techniques capable of identifying VOCs have been developed during the time [28]. However, these methods present different limits of detection, sensitivity, and type of detection, those based on mass spectrometry (MS), seem to be more reliable (**Table 1**). Nevertheless, these methods are pretty expensive and difficult to be implemented in daily practice. They are mostly used to evaluate the best mixture of VOCs detected in the breath that can then be used for the development of portable sensors [29]. Accordingly, in one study, by combining GC-MS with support vector machine assessment, the Sakumura's group pointed out a mixture of five VOCs, including, isoprene, CHN, 1-propanol, CH_3CN, and methanol, correlated with lung cancer [30]. Even though the most reliable VOCs analysis method is GC-MS, data analysis can be provided by multiple algorithms, such as: partial least-squares regression (PLS) [31], forward stepwise discrimination (FSD) [32, 33], weight digital sum discriminator [34], logistic regression [35], random forest classification (RFC) [36], linear canonic discriminant analysis (CDA) coupled with principal component analysis (PCA)

Table 2. Advantages and disadvantages of e-noses techniques of analysis

Analysis Techniques	Advantages	Disadvantages	Bibliography
colorimetric sensor assays	Fast and cheap Easy to use Small sample size Customization for specific analysis Versatile-liquid or gas samples can be integrated in smart phones	Reproducibility of printing and imaging Stability Difficult to determine the exact composition of a mixture Extensive data set analysis using chemometric methods	[55]
conductive polymer gas sensors	Easy to adjust the sensitivity of the sensor Operated at room temperature High sensitivity Easy fabrication	Long time storage in the air makes them instable Low selectivity	[56]
quartz microbalance sensor array	Robust performance Simple construction Sensitive detection Versatile	Moderate reproducibility Complex manufacture Sensitive to humidity and temperature	[57]
metal oxide sensors	High sensitivity Rapid response	High-temperature operation Sensitive to sulfur, low acids, and humidity Limit sensor coating Low precision	[58]

[37], that could lead to different results. Meaning, using PLS, which is a linear chemometric test, the obtained results may be underestimated and better results can be provided using nonlinear methods [31]. In the case of FSD, this analysis is based on the assumption of a multivariant normal distribution, and in most of the case, the analyzed subgroups are smaller than the starting group but the performance is similar [38]. RFC is a method based on tree-based algorithms and uses several decision trees used from randomly selected subsets [39]. PCA is used to transform observations, characterizing different variable into a smaller set of values uncorrelated to variables. CDA uses linear, orthogonal transformation of a set of observed variable, when we compare the two methods CDA gives better results [40]. Recently, some new approaches to analyze VOCs with good results have been developed. One example is given by electric noses, which can employ different techniques for analysis, as colorimetric sensor assays [35, 41, 42], conductive polymer gas sensors [37, 43, 44], quartz microbalance sensor array [31, 45, 46], metal oxide sensors [47, 48] and gold nanoparticle sensor array [49-51]. Some advantages and disadvantages of these techniques are presented in **Table 2**.

VOC analysis was applied by several studies that provided different conclusions about their accuracy to diagnose lung cancer. Some study says that increase concentration of xylene in cancer patients is correlated to smoking [59]. Others say that this is correlated with the progression of lung cancer and shows no correlation to smoking status [60]. However, even if VOCs seems to be the same between healthy people and lung cancer patients, their concentration is different, for example, the propane concentration in a healthy individual is between 3.45-5.96 ppb, while in lung cancer patients is 3.19-9.74; hexane 1.75-6.31 ppb and 0.82-1.88 ppb, respectively [61-63]. As can be seen, the concentration of VOCs, are very different between each cancer patient and control individual, and in some part they overlay, which makes it hard to choose to find one VOC that could discriminate between cancer and healthy persons. Moreover, as can be observed in **Table 3** a mixture of VOCs could increase the accuracy of lung cancer diagnosis, when compared with individual VOC, but still, there is not a standardized mixture observed in all tested patients, and the values differ from study to study.

One major disadvantage of this method is the ranges of concentrations of VOCs that can be obtained both for cancer patients and controls and also the fact that in some cases the ranges for cancer patients overlap with those for healthy individuals (see also **Table 3**). As can be observed from the data presented in **Table 3**, if there is an increased number of VOCs tested, the specificity and the sensitivity of diagnosis lung cancer is pretty high. A major disadvantage is due to the fact that there are no standardized regulations for VOCs analysis regarding units for concentration, and also, there is not a reference value for each VOC in

Table 3. The mixture of VOCs correlated to lung cancer and the specificity and sensitivity of the group of VOCs to discriminate between patients with cancer and healthy individuals

VOCs	Value (concentration in controls (ctr) and cancer patients (cc)	Unit	Sensitivity (%)	Specificity (%) (AUC)	Ref
Butane	NA	NA	89.6	82.9	[33]
3-methyl Tridecane	NA	NA			
7-methyl Tridecane	NA	NA			
4-methyl Octane	NA	NA			
3-methyl Hexane	NA	NA			
Heptane	NA	NA			
2-methyl Hexane	NA	NA			
Pentane	NA	NA			
5-methyl Decane	NA	NA			
Isoprene	Ctr-3.789, cc-6.041	nmol/L	72.2	93.6	[64]
Methylpentane	Ctr-0.277, cc-1.39	nmol/L			
Pentane	Ctr-0.268, cc-0.647	nmol/L			
Ethylbenzene	Ctr-0.013, cc-0.024	nmol/L			
Xylenes	Ctr-0.031, cc-0.068	nmol/L			
Trimethylbenzene	Ctr-0.006, cc-0.014	nmol/L			
Toluene	Ctr-0.0808, cc-0.158	nmol/L			
Benzene	Ctr-0.044, cc-0.094	nmol/L			
Heptane	Ctr-0.008, cc-0.013	nmol/L			
Decane	Ctr-0.208, cc-0.568	nmol/L			
Styrene	Ctr-0.012, cc-0.017	nmol/L			
Octane	Ctr-0.020, cc-0.061	nmol/L			
Pentamethylheptane	Ctr-0.0009, cc-0.002	nmol/L			
Isobutane	cc-6.48	ppbv	71.4	91.9	[43]
Methanol	cc-84	ppbv			
Ethanol	cc-603	ppbv			
Acetone	cc-321.25	ppbv			
Pentane	cc-1.875	ppbv			
Isoprene	cc-103	ppbv			
Isopropanol	cc-438.75	ppbv			
Dimethylsuflide	cc-1.37	ppbv			
Carbon disulfide	cc-1.9	ppbv			
Benzene	cc-2.6	ppbv			
Toluene	cc-4.27	ppbv			
Cyclododecatriene	0.57	AUC	84.6	80	[65]
Pentane	0.69	AUC			
Benzoic acid	0.69	AUC			
Propanoic acid	0.77	AUC			
azepine	0.77	AUC			
Cyclohexadiene	0.8	AUC			
Benzene	0.79	AUC			
Furan	0.79	AUC			
Biphenyl	0.79	AUC			
Pentanone	0.8	AUC			
Caryophyllene	0.8	AUC			
Indene	0.87	AUC			

Propanol	0.87	AUC			
Decane	0.86	AUC			
Benzenedicarboxylic acid	0.87	AUC			
Hexadiene	0.9	AUC			
Benzene	Ctr-2.4, cc-2.9	ppb	71	100	[66]
isoprene	ctr-105.2, cc-81.5	ppb			
acetone	ctr-627.5, cc-458.7	ppb			
methanol	ctr-142, cc-118.5	ppb			
Pentanal	Ctr-0.002, cc-0.019	nmol/L	75	95.8	[67]
Hexanal	ctr-0, cc-0.01	nmol/L	8.3	91.7	
Octanal	Ctr-0.011, cc-0.052	nmol/L	58.3	91.7	
Nonanal	Ctr-0.033, cc-0.239	nmol/L	33.3	95.8	
Hexadecanal	0.949	AUC	96.47	97.47	[68]
2,6,10,14-tetramethylpentadecane	0.936	AUC			
Eicosane	0.828	AUC			
5-(2-methyl) propylnonane	0.8	AUC			
7-methylhexadecane	0.754	AUC			
8-methylheptadecane	0.743	AUC			
2,6-di-tert-butyl, 4-methylphenol	0.738	AUC			
2,6,11-trimethyldodecane	0.719	AUC			
3,7-dimethylpentadecane; nonadecane	0.708	AUC			
8-hexylpentadecane	0.674	AUC			
2,6,10-trimethyltetradecane	0.661	AUC			
5-(1-methyl-) propylnonane	0.659	AUC			
2-methylnapthalene	0.658	AUC			
2-methylhendecanal	0.653	AUC			
nonadecanol	0.646	AUC			
2-pentadecanone	0.640	AUC			
3,7-dimethyldecane	0.638	AUC			
tridecanone	0.627	AUC			
5-propyltridecane	0.623	AUC			
2,6-dimethylnaphthalene	0.618	AUC			
tridecane	0.616	AUC			
2,8-dimethylhendecane	0.613	AUC			
5-butylnonane	0.604	AUC			
Dodecane	NA	NA	76	100	[69]
Butanol	NA	NA			
Metylbutylacetat	NA	NA			
Hexanol	NA	NA			
Cyclohexanon	NA	NA			
Iso-propylamin	NA	NA			
nNonal	NA	NA			
Cyclohexanon	NA	NA			
Ethylbenzol	NA	NA			
Hexanal	NA	NA			
Heptanal	NA	NA			
Pentane	Ctr-5.1, cc-6.6	ng/l	80	90	[70]
Hexane	ctr-0.8, cc-1.2	ng/l			
Heptane	ctr-1, cc-1	ng/l			

Octane	Ctr-0.9, cc-1	ng/l
Dodecane	Ctr-1.5, cc-2	ng/l
2-Methylpentane	Ctr-1.9, cc-1.7	ng/l
3-Methylpentane	Ctr-0.9, cc-0.8	ng/l
Cyclohexane	Ctr-4.8, cc-1.7	ng/l
Benzene	Ctr-0.3, cc-3.4	ng/l
Ethylbenzene	Ctr-0.5, cc-1.4	ng/l
Propylbenzene	Ctr-0.17, cc-0.33	ng/l
Propanal	Ctr-18.3, cc-58.5	ng/l
Butanal	Ctr-0.5, cc-1	ng/l
Pentanal	Ctr-0.7, cc-0.9	ng/l
Hexanal	ctr-3, cc-3.3	ng/l
Octanal	Ctr-0.5, cc-0.9	ng/l
Nonanal	ctr-1.3, cc-2	ng/l
Decanal	Ctr-0.8, cc-1.9	ng/l
Butanol	Ctr-1.9, cc-6.4	ng/l
Butanone	Ctr-4.8, cc-6.9	ng/l
Pentanone	Ctr-3.1, cc-2.8	ng/l
Isoprene	Ctr-730, cc-720	ng/l
Acetone	Ctr-9900, cc-7980	ng/l
2-Propanol	Ctr-3750, cc-6070	ng/l

NA - not available; AUC - area under the ROC (receiver operating characteristic curve) curve.

control individuals which makes it harder to discriminate between cancer samples and controls.

Exhaled breath condensate analysis

Exhaled breath condensate (EBC) is obtained by cooling the exhaust air from patients. These approaches represent useful tools for monitoring diseases that are related to oxidative stress. By EBC, it is possible to evaluate the presence of aldehydes, peroxide, leukotriene, cytokines, and adenosine, which are essential biomarkers in several different diseases including lung cancer [71-74]. Nunez-Naveira et al. observed that dermcidin or proteolysis-inducing factor (PIF) and S100A9 could be potential biomarkers for disease progression as they can be detected in EBC. They are overexpressed in lung cancer samples, and they are correlated with lung cancer development [75]. Previous data have pointed out specific EBC profiles for lung cancer patients, and that these metabolite profiles could discriminate between different stages of lung cancer. Accordingly, Peralbo-Molina et al. observed that several specific metabolites (13-Heptadecyn-1-ol, Monopalmitin, n-Hexadecylindane, Monostearin, and Squalene) can differentiate between lung cancer patients and risk factor patients [76]. Moreover, they observed that other set of BEC metabolites including cumyl alcohol, benzoic acid methyl ester, 2,4,6-triisopropylphenol, 2,6-bis (1,1-dimethylethyl)-4-(1-methyl-1-phenylethyl) phenol, 2,4-bis (1-methyl-1-phenylethyl) phenol and 2,4-bis (dimethylbenzyl-6-t-butylphenol can discriminate between lung cancer patients stage I+II, III+IV and patients with risk of lung cancer [77]. In another study, Ahmed et al. observed that lung cancer patients have higher concentrations of propionate, ethanol, acetate, and acetone but lower concentrations of methanol than patients with benign conditions [78]. There are several other studies that present de use of EBC in lung cancer diagnosis, progression or treatment evaluation (**Table 4**).

Till now, several commercially available portable devices for EBC collection, including EcoScreen, Turbodeccs, Rtube, and Anacon glass condenser have been developed [79]. While unsupervised subjects at home could use EcoScreen, TuboDECCS, RTube, ANACON condenser is used mainly for EBC collection from patients that are mechanically ventilated,

Table 4. Studies presenting the use of EBC in lung cancer (year of publication 2015-2019)

EBC collection device	Study population	Type of analysis performed	Ref
EcoScreen-1 (Erich Jaeger GmbH, Hochberg, Germany) with saliva trap	51 patients with a diagnosis of NSCLC	KRAS mutations identification	[80]
Rtube condensate collector device (Model Austin, TX 78720; RTube starter kit; Respiratory Research Inc., Austin, TX)	60 patients with lung cancer who underwent lung resection	Evaluation of TNF-α, IL-1β expression levels	[81]
RTube condenser (Model Austin TX 78720; RTube starter kit; Respiratory Research Inc., Austin, TX, USA)	60 patients with lung cancer undergoing a unilateral lobectomy	Evaluation of surfactant protein A and surfactant protein D expression levels	[82]
EcoScreen 2 device (FILT Lungen-und Thoraxdiagnostik, Berlin, Germany)	192 individuals the control group *n* = 49; smoking group *n* = 49 the chronic obstructive pulmonary disease group *n* = 46; lung cancer group *n* = 48	Proteomic analysis	[83]
RTube (Respiratory Research)	61 ctr and 50 NSCLC and 1 SCLC-FFPE Tissue 143 ctr, 99 NSCLC, and 9 SCLC-EBC samples	Evaluation of *GATA6* and *NKX2-1* expression	[84]
HAAK EK20 EcoScreen; Eric Jaeger, Friedberg, Germany	58 patients with NSCLC, 30 healthy subjects were selected	Evaluation of the mutational status of exons 1 and 2 of the p16 gene	[85]

because it can be wired to the ventilation machine.

The studies presented in **Table 4** use different types of instruments for EBC collection but they are able to demonstrate that the EBC samples can be used for diagnosis of lung cancer or chronic obstructive pulmonary disease (COPD). Kordiak et al. demonstrated that *KRAS* mutations identified in EBC samples are the same as in the case of the tissue samples, and from the 12 positive tissue sample, 11 samples presented mutations also in EBC samples, demonstrating that EBC analysis has high sensitivity and specificity [80]. Sanchez et al. analyzed the proteomic profile of EBC samples from lung cancer patients and observed that the amounts of proteins are increased in EBC samples during tumorigenesis and that lung cancer EBC samples have high levels of dermidin, hemoglobin, histones and cytokeratins and low levels of hornerin. Also, they developed a diagnosis model using Random forest model and observed an AUC of 82% for the control group, 76 for high-risk factors like smoking, and 77% for COPD [83]. Chen et al. was able to obtain a mutation rate for p16 of 14.81% in EBC samples from NSCLC patients, and no mutation could be identified in healthy control patients, which means that this method could be used as a noninvasive diagnosis method for NSCLC. Also, they observed that the mutation rate was increased with the increased tumor stage [85]. All these studies presented above used the EcoScreen devices, but there are also studies that used the Rtube condensers and still manage to obtain good results. An example in this way presented by Lin et al. which observed that the expression of TNFα and IL-1β in EBC samples from COPD patients was correlated to their mRNA expression in tissue and lung inflammation. They observed that the levels of TNFα and IL-1β decreased after lung resection and lung-protective treatment and that these levels are correlated with lung function [81]. Another study on COPD patients observed that surfactant protein A (SP-A) and D (SP-D) levels are decreased in COPD EBC and mRNA samples, and they increase after treatment, showing a good correlation between EBC and mRNA analysis. Expression levels of SP-A and SP-D were also correlated with lung function which could make them useful biomarkers for COPD diagnosis. Mehta et al. were able to successfully use EBC samples for evaluation of Em and Ad isoforms of *GATA6* and *NKX2-1* genes and observed that the Em/Ad ratio of *GATA6* and *NKX2-1* genes was increased in lung cancer patients than in controls, but they could not identify these isoforms in NSCLC patients, so the two biomarkers could be used in lung cancer patients diagnosis, except for NSCLC patients [84]. So, it has been shown that independent of the instrument used to performed EBC sample collection, the results described EBC as a promising non-invasive method for either lung cancer or COPD diagnosis, no matter what type of analysis you intend to do either genomic, transcriptomic or proteomic.

Genomic methods for lung cancer diagnosis

As we mention above the VOCs and EBS analysis has brought important developments and benefits into lung cancer screening and still there is a lack of knowledge in early diagnosis of this cancer. This issue could be overcome by genomic approaches that provide higher sensitivity and specificity than VOCs and EBS, even though a small amount of sample is used. Through genomics approach, genetic/genomics alterations associated with cancer phenotype can be identified and detected even before the onset of clinical symptoms. One promising approach is the use of epigenetic or gene expression biomarkers specific from sputum or bronchial aspirate [86, 87]. Moreover, predictive models that include such biomarkers could be used in the clinic in order to assess the risk of each individual, which could be translated into personalized medicine [88].

In their study, Diaz-Lagares et al. were able to obtain an epigenetic signature characteristic for stage I lung cancer in formalin-fixed paraffin-embedded tissue (FFPE), and then they validated their results in sputum, bronchial aspirate, and bronchoalveolar lavages. They observed that in lung cancer samples, the *BCAT1, CDO1, TRIM58, ZNF177*, and *CRYGD* genes are hypermethylated and the information from the TCGA database correlate their low expression with the methylation status. Also, they revealed higher diagnostic efficiency in the bronchial liquid that in conventional cytology [89]. Another study observed that

Table 5. Molecular studies of lung cancer biomarker identification (year of publication 2019-2015)

Authors	Patients	Sample type	Biomarkers identified	The subtype of lung cancer	Sensibility and specificity/AUC
Hunag [99]	65 lung cancer patients and 10 healthy controls	Plasma and tissue	Tissue Methylation profile: *CDKN2A 57% (13/23), DLEC1 65% (15/23), CDH1 48% (11/23), DAPK 74% (17/23), RUNX3 57% (13/23), APC 48% (11/23), WIF1 39% (9/23), and MGMT 4% (1/23)* Plasma Methylation profile: 45% for *CDKN2A, 48% for DLEC1, 76% for CDH1, 14% for DAPK, 29% for RUNX3*	AD-32 SC-18 ASC-15	At least one gene affected of the eight studies: 95% and 100% At least two genes affected of the eight studies: 71% and 100% At least three genes affected by the eight studies: 40% and 100%
greZhang [100]	66 NSCLC patients and 67 healthy controls	Plasma	Methylation gains in *SIPA1L2 RSPO3, LDB2, ZNF679, AP001604.3*, and *RP1-137K24.1.*	AD-46 SC-17 ASC-3	0.96
Zare [101]	60 lung cancer patients and 20 healthy controls	Whole Blood	*HERV-R, HERV-H, HERV-K, and HERV-P* eng genes	AD-38 SC-10 SCLC-10	NA
Peng [102]	Lung adenocarcinoma (LUAD) patients from several studies-meta analysis	Different types of samples	miR-21-5p, miR-210-3p, miR-182-5p, miR-183-5p, miR-126-3p and miR-218-5p	AD	NA
Hocker [103]	40 stage I lung cancers and 40 controls	Serum	Mass peak profiling	AD-20 SC-20	95% and 85/0.95
Wang [104]	Lung cancer patients from seven studies-meta-analysis	Tissue	miR-21-5p and miR-223-3p, miR-126-3p, miR-133a-3p, miR-140-5p, miR-143-5p, miR-145-5p, miR-30a-5p, miR-30d-3p, miR-328-3p, miR-451	NA	NA
El-Zein [105]	216 SCLC, 196 NSCLC, 229 healthy controls	Blood	when binucleated cells with micronuclei [BN-MN], nucleoplasmic bridges [BN-NPBs], and nuclear buds [BN-BUDs]	SCLC-216 NSCLC-196	NA
Sui [106]	463 AD patients from TCGA database, 53 additional AD patients	tissue	miR-30a-3p, miR-96-5p and miR-182-5p	AD-516	0.837 for *miR-30a-3p* 0.819 for *miR-96-5p* and 0.835 for *miR-182-5p*
Codreanu [107]	Discovery set: 34 benign lung nodules, 24 untreated AD, and 10 biopsies of bronchial epithelium Validation set: 20 benign nodules, 21 AD, and 20 normal bronchial biopsies	tissue	ALOX5, ALOX5AP, CCL19, CILP1, COL5A2, ITGB2, ITGAX, PTPRE, S100A12, SLC2A3CEACAM6, CRABP2, LAD1, PLOD2, and TMEM110-MUSTN1	AD-44	0.52-0.99
Imperatori [108]	167 early stage NSCLC patients	Tissue	*LINE-1* hypermethylation	AD-100 SC-67	NA
Pamungkas [109]	15 NSCLC patients, with (n=10) and without (n=5) EGFR mutations	Plasma	linoleic acid, tetradecanoyl carnitine, 5-methyl-tetrahydrofolate (-MTHF), and N-succinyl-L-glutamate-5 semialdehyde (NSGS)-	NA	46.67%, 86.67%, 0.68-linoleate 43.33%, 100%, 0.72-5MTHF 46.67%, 86.67%, 0.65-NSGS 63.33%, 73.33%, 0.69-tetradecanoyl carnitine
Jung [110]	Training set-75 NSCLC patients and 75 controls Validation-25 primary lung cancer patients and 25 controls	Serum	EGFR1, MMP7, CA6, KIT, CRP, C9, and SERPINA3	AD-70 SC-29 Large cell carcinoma-1	EGFR1-0.69, MMP7-0.61, CA6-0.7, KIT-0.56, CRP-0.66, C9-0.73, SERPINA3-0.66

Wang [111]	200 patients with primary lung cancer and 200 controls with no cancer	Blood	alteration in the methylation profile of *p16*, *RASF1A* and *FHIT* and relative telomere length (RTL)	NA	82% 80% 0.81
Hulbert [112]	150-early-stage lung cancer patients and 60 controls	Plasma and sputum	Methylation profile of *SOX17, TAC1, HOXA17, CDO1, HOXA9*, and *ZFP42*	AD-121 SC-26 ASC-3	93%, 86%, 0.85 for sputum 93%, 67%, 0.89 for plasma
Baglietto [113]	Discovery set: 552 case-control pairs Validation set: 429 case-control pairs	Blood and dried blood spots	DNA methylation (6 CpGs) profile	NA	0.826
Widlak [114]	95 early stage lung cancer patients and 285 healthy controls	Serum	compounds with m/z 1759.5, 1867.0, 3018.9, 3308.4 and 9553.8 Da Da	AD-58 SC-35 NSCLC-2	Discovery set: 100%, 63%, 0.88 Validation set: 86%, 34%, 0.734
Huang [115]	20 patients with early stage NSCLS and 10 healthy controls	Plasma	miR-148a, miR148b and miR-150	AD-10 SC-10	NA
Fahrmann [116]	29-benign nodules patients 17-lung cancer pre-diagnosis samples 25-lung cancer at diagnosis 19-lung cancer post-diagnosis	Serum	phosphatidylethanolamines (PE34:2, PE36:2 and PE38:4)	AD-46 SC-5 SCLC+AD-1 SCLC-6	PE34:2-0.8 PE36:2-0.77 PE38:4-0.79
Jin [117]	Test set: 6-healthy subjects; 6-patients with benign tumors; 9-patients with IA NSCLC; 4 IB NSCLC Validation: 19 healthy subjects; 25 patients with benign tumors; 60 NSCLC patients	Serum	GlcNAcylated AACT and CEA	NA	64.8% 93.1% 0.817
Powrozek [118]	65 lung cancer patients, 95 healthy subjects	Plasma	*DCLK1* promoter methylation profile	AD-22 SC-20 Large cell carcinoma-4 SCLC-19	NA
Fahrmann [119]	Training set: 52 AD and 31 controls Validation set: 43 AD and 43 controls	Serum and Plasma	Aspartate, glutamate, and Bin_225393, Pyrophosphate, maltotriose, citrulline, adenosine-5-phosphate, Bin_226841, and Bin_36799	AD-95	Serum-0.86 Plasma-0.88
Wikoff [120]	39 AD patients	tissue	ribitol, arabitol, UDP-N-acetylglucosamine (UDP-GlcNAc), xylitol, fucose/rhamnose and glucose	AD-39	NA
Kim [121]	72 NSCLC patients and 30 healthy subject	Plasma	Alpha-actinin-1, Fructose-bisphosphate aldolase A, Alpha-enolase, Filamin-A, Glucose-6-phosphate 1-dehydrogenase, Glucose-6-phosphate isomerase, Endoplasmin, Intercellular adhesion molecule 1, Integrin-linked protein kinase, L-lactate dehydrogenase B chain, Moesin, Phosphoglycerate kinase 1, Pyruvate kinase isozymes M1/M2, Osteopontin, Transaldolase, Thrombospondin-1, Zyxin	AD SC Large cell carcinoma	Zyxin-0.958

AD - adenocarcinoma, SC - squamous cell carcinoma, ASC - adenosquamous carcinoma, SCLC - small cell lung cancer, NA - not available, NSCLC - non-small cell lung cancer.

TMEM196 methylation is an independent biomarker correlated with prognostic of lung cancer patients, in early stages. Also, this biomarker can be detected in both plasma and sputum samples of lung cancer patients with high specificity and sensitivity [90]. Tomasseti et al. observed that *p16INK4A, RARB2, RASSF1A*, and *SOX17* gene are aberrantly methylated in circulating free DNA samples of lung cancer patients [91]. Also there are studies that correlate different microRNAs (miRNAs) with lung cancer diagnosis, prognosis, or treatment efficacy [92]. In this way, miR-21, miR-183 family, miR-126 and miR-155, were correlated with poor prognosis and short survival in lung cancer [93-96]. Boeri et al. observed that miRNAs analysis of plasma samples from lung cancer patients could be used to identify biomarkers for diagnosis and prediction with specificity compared with CT scans [97], while Sozzie et al. described a miRNA plasma signature of lung cancer patients that could reduce the false-positive results of low-dose CT [98].

There are also several studies that correlate methylation, gene expression or miRNAs profiles with lung cancer and that identify different biomarkers that could be used in early detection of lung cancer. The studies presented in **Table 5** use different biological samples and apply different molecular technologies in order to discover the best diagnostic tool for lung cancer. The evaluation of methylation or proteomic profile of lung cancer patients shows the best result in discriminating between lung cancer and healthy patients as can be seen by the sensitivity and specificity of the experiments presented in **Table 5**.

Several different biomarkers were used in different studies in order to discriminate between lung cancer patients and healthy individuals, but as can be seen in **Table 5**, the best test is based on the evaluation of the methylation profile of these two groups of patients and also on the proteomic analysis.

In the case of methylation analysis we can see that this is a versatile method that was employed with success and showed promising result in lung cancer early diagnosis in patients that are exposed to different environmental pollutants like smoky coal [99], or for the evaluation of different biomarkers that could help early diagnosis of lung cancer or predict recurrence even in early stages of NSCLS cancer, like hypermethylation of LINE-1 [108], *SIPA1L2 RSPO3, LDB2, ZNF679, AP001604.3*, and *RP1-137K24.1* [100], *SOX17, TAC1, HOXA17, CDO1, HOXA9*, and *ZFP42* [112] *or DCLK1* [118]. In other study the methylation profile of lung cancer patients and healthy controls have been associated with different algorithms, like support vector machines (SVMs) or decision trees (DTs), for better discrimination of lung cancer patients [111].

As a result of the numerous studies in this field, one can say that in order to achieve 100% specificity and 100% sensibility of a test for discrimination of lung cancer and possible an early diagnosis tool for it we need to develop a test that will combine methylation profiles, proteomic analysis, gene and miRNAs expression, and mutation status results.

In conclusion, there are a lot of studies dealing with the early diagnosis of lung cancer, but still, there is an increasing percentage of death from this type of cancer. As described in this article, it is possible that a combination of methods could have better discrimination and also could help clinicians performed a more specific and less invasive diagnosis than just employing just one of the diagnostic tools described here.

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Disclosure of conflict of interest

None.

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