REVIEW

Exhaled breath analysis for the early detection of lung cancer: recent developments and future prospects

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Abstract: In lung cancer, the prognosis and treatment options depend directly on tumor size and its spread at the time of diagnosis. There is therefore a constant search for methods that will allow early detection of cancerous lung nodules. With advancing imaging technology and implantation of screening routines in high-risk populations by low-dose computerized tomography, a significant increase in the number of diagnosed small peripheral lesions can be expected. While early detection of small cancerous lesions carries the benefit of wider treatment options and better prognosis, the process of obtaining a biopsy to confirm a cancerous tissue is not free of complications and bears inconveniences and stress to the patient. This review discusses the potential use of exhaled breath analysis as a simple, noninvasive tool for early detection of lung cancer and characterization of suspicious lung nodules.

Keywords: breath sampling, volatile organic compounds, lung cancer, early detection, electronic nose

Introduction

Lung cancer (LC) is the second most prevalent cancer in adult men and women around the world, but it is also the deadliest in both sexes.[1] The prognosis and treatment options of LC patients depend directly on tumor size and its spread at the time of diagnosis; survival time decreases significantly as the disease is more progressive at detection.[2] Screening and early detection through low-dose computerized tomography (LDCT) reduces LC-related mortality by 20%.[3,4] Therefore, screening programs are supported now in the US and also recommended by the European societies.[5] However, there are still obstacles in the implementation of the screening programs, mainly due to lack of infrastructure to handle high volume of patients with pulmonary findings. Therefore, the unmet current clinical need is to determine the relevant noninvasive biomarkers that may support this effort.[6] Here, we review the use of exhaled breath analysis as a potential candidate for such important purpose.

There is a longstanding interest in the potential of using exhaled breath analysis for detection of various diseases, apart from LC detection, which is reviewed in detail in this article. In a most recent publication, Nakhleh et al[7] used an artificially intelligent nanoarray sensor in the diagnosis and classification of 17 different diseases from breath samples of 1404 subjects, with 86% accuracy. The same system was able to detect LC in patients with lung nodules,[8] detect response to therapy and early recurrence[9] and further specify sub-histology of LC[10] and even genetic mutation.[11] Interestingly, Peng et al[12] showed that this system was able to discriminate four types of primary cancers

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(lung, breast, colorectal and prostate). This review focuses on the ongoing research of exhaled breath analysis for the early detection of LC.

Nature of volatile organic compounds (VOCs)

In addition to oxygen, nitrogen and carbon dioxide, the exhaled breath contains low concentrations of various VOCs. Most VOCs are believed to reflect endogenous metabolic processes at the tissue level, such as inflammation and oxidative stress. However, breath analysis also detects exogenous VOCs, which reflect exposure to carcinogens such as cigarette smoke, pollution and radiation. These VOCs are typically highly reactive substances that damage DNA and proteins, a process that over time might promote cancerous changes in various tissues. When cancer develops, various inflammatory processes as well as gene and protein changes take place; these metabolic changes can create a unique VOC profile that is potentially reflected in the body fluids. As various VOCs reach the lungs through blood stream, they are then excreted by diffusion across the pulmonary alveolar membrane and exhaled through the breath.[10,13,14]

Although almost 3000 VOCs have been reported in scientific literature, their majority can be divided into few main groups: 1) hydrocarbons – produced mainly by peroxidation of polyunsaturated fatty acids; 2) alcohols – absorbed through the gastrointestinal tract and metabolized mainly in the liver;

3) aldehydes – arise from metabolized alcohols/lipid peroxidation/tobacco smoke/dietary sources; 4) ketones – produced from fatty acids by the liver and influenced by increased fat or protein intake in diet and 5) aromatic and nitrile VOCs – typically considered as arriving from exogenous pollutants such as tobacco smoke and pollution.[15]

A wide range of modified nanomaterials has been used in exhaled breath analysis, such as nanoparticles (NPs), silicon nanowires (SiNWs) and carbon nanotubes (CNTs). Two main approaches are used in the detection and characterization of VOCs. The first is selective detection of specific VOCs, with gas chromatography being the most commonly used technique. This approach is based on using highly selective receptors designed for pre-identified and selected VOCs. The main limitation of this approach, as will be discussed later, is that currently there are no VOCs that are identified as unique for a specific disease. The second approach is cross-reactive nanomaterial-based sensor arrays, which allows pattern recognition rather than specific VOC identification. There are few techniques that use this approach; the main one is based on electrical conductivity – the electronic nose (eNose). Another technique is the use of colorimetric sensors, which chemically react and change colors upon exposure to different VOCs.[15,16] The following sections give a further insight to studies conducted in each of the described approaches. Table 1 presents a selection of studies reporting breath test performance for LC detection.

Table 1 A selection of studies reporting breath test performance for LC detection

Reference	Participants	Technique	Results		
			Sensitivity (%)	Specificity (%)	Accuracy (%)
GC-MS					
Gordon et al, 1985[18]	12 PLC patients 17 HCs	TD-GC-MS	NR	NR	93 (3 VOCs) 100 (22 VOCs)
Phillips et al, 1999[19]	60 PLC patients 48 HCs	TD-GC-MS	71.7	66.7	69.4
Phillips et al, 2003[20]	178 bronchoscopy patients (67 PLC patients) 41 HCs	TD-GC-MS	85.1	80.5	83.3
Poli et al, 2005[21]	36 NSCLC patients 110 controls (25 mild-to-moderate COPD patients, 35 smokers with no COPD and 50 nonsmokers)	SPME/TD-GC-MS	72.2	93.6	88.4
Phillips et al, 2007[23]	193 PLC patients 211 HCs	TD-GC-MS	84.6	80.0	NR
Phillips et al, 2008[24]	193 PLC patients 211 HCs	TD-GC-MS	84.5	81.0	
Bajtarevic et al, 2009[25,#]	65 PLC patients 31 HCs	SPME/GC-MS	52 71 80	100 (4 VOCs) 100 (15 VOCs) 100 (21 VOCs)	NR

(Continued)

Table 1 (*Continued*)

Reference	Participants	Technique	Results		
			Sensitivity (%)	Specificity (%)	Accuracy (%)
Ligor et al, 2009[26]	65 PLC patients 31 HCs	SPME/GC-MS	51	100	NR
Poli et al, 2010[22]	40 PLC patients 38 HCs	SPME/GC-MS	90	92.1	91.0
Wang et al, 2012[30]	88 PLC patients 155 controls (70 benign lung disease patients and 85 HCs)	SPME/GC-MS	96.5	97.5	97.1
Phillips et al, 2015[38]	96 PLC patients 205 controls	GC-MS	74.0	70.7	NR
Schallschmidt et al, 2016[32]	37 PLC patients 23 HCs	SPME/GC-MS	80	90	NR
eNose					
Di Natale et al, 2003[39]	35 PLC patients 18 controls 9 after surgical therapy	LibraNose	NR	NR	100
Machado et al, 2005[40]	First phase: 14 bronchogenic carcinoma patients and 45 HCs Second phase: 14 PLC patients and 62 HCs	Cyranose 320	71.4	91.9	NR
Mazzone et al, 2007[46]	49 PLC patients 63 other lung diseases patients 21 controls	Colorimetric sensors	73.3	72.4	NR
Dragonieri et al, 2009[42]	10 PLC patients 10 HCs	Cyranose 320			90
	10 PLC patients 10 COPD patients				85
D'Amico et al, 2010[50]	28 PLC patients 36 HCs	QMS sensors	85	100	NR
	28 PLC patients 28 other lung disease patients		92.8	78.6	85.7
Santonico et al, 2012[55]	20 PLC patients 10 controls	QMS sensors	85	85	85
Hubers et al, 2014[56]	20 PLC patients 31 COPD controls Validation: 18 PLC patients and 8 HCs	Cyranose 320	80	48	
McWilliams et al, 2015[43]	25 PLC patients 166 controls	Cyranose 320	NR	NR	80
Gasparri et al, 2016[44]	70 PLC patients 76 HCs	QMS sensors	81	91	NR
Nisreen et al, 2016[57]	149 PLC patients 56 controls (COPD/asthma)	SiNW FET	87	82	84
Both eNose and GC-MS					
Peled et al, 2012[8]	53 PLC patients 19 benign nodules	NaNose, SPME/GC-MS		96	88
Broza et al, 2013[51]	12 PLC patients 5 benign nodules	NaNose, SPME/GC-MS	100	80	94.1
Capuano et al, 2015[54]	20 PLC patients 10 other lung diseases patients	NaNose, SPME/GC-MS	NR	NR	90 (NaNose) 76 (SPME/GC-MS)

Note: #Data is given for 3 separated analyses. Studies were included if sensitivity, specificity or accuracy was specified in their text.
Abbreviations: COPD, chronic obstructive pulmonary disease; eNose, electronic nose; FET, field effect transistors; GC-MS, gas chromatography–mass spectrometry; HC, healthy control; LC, lung cancer; NaNose, Nanoscale Artificial Nose; NSCLC, non-small cell lung cancer; NR, not reported; PLC, primary lung cancer; QMS, quadrupole mass spectrometry; SiNW, silicon nanowire; SPME, solid phase microextraction; TD, thermal desorption; VOC, volatile organic compound.

Gas chromatography–mass spectrometry (GC-MS)

In the GC-MS method, exhaled breath is collected and temporarily stored in designated containers (e.g., in inert bags or sorption tubes). Then, a helium stream is used to carry the sample though a long, heated capillary column. The VOCs are separated based on their chemical properties, consecutively ionized and separated by their mass/charge (*m/z*) ratio and are identified by a spectral library in the GC software.[17]

Back in 1985, Gordon et al examined breath samples of 12 LC patients and 17 healthy volunteers. A total of 22 VOCs that showed the largest difference between the two groups were recognized. In further analysis, three VOCs were chosen according to their peak and occurrence in the subjects, including acetone, methyl ethyl ketone and *n*-propanol. Using these three VOCs, the authors accurately classified 93% of the samples.[18]

Phillips et al collected breath samples from 108 patients with an abnormal chest radiograph who were scheduled for bronchoscopy. LC was confirmed histologically in 60 patients. Analyzing the breath samples, a combination of 22 VOCs discriminated between patients with and without LC, regardless of stage. For stage 1 LC, the 22 VOCs had 100% sensitivity and 81.3% specificity.[19] In a different study, the same authors reported identifying primary LC with a sensitivity of 89.6% and a specificity of 82.9% using nine VOCs that were chosen after comparing primary LC patients with healthy controls. The authors noted that patients with primary LC had breath test findings that were consistent with the accelerated catabolism of alkanes and monomethylated alkanes.[20]

Poli et al chose 13 VOCs to compare three groups, patients with non-small cell lung cancer (NSCLC), subjects with mild–moderate chronic obstructive pulmonary disease (COPD) and asymptomatic smokers as controls. None of the selected VOCs alone distinguished the NSCLC patients from the other study groups, but overall VOC concentrations were highly discriminant (>70%).[21] In a later study, the same authors measured exhaled aldehydes as a biomarker of NSCLC; the levels of all aldehydes in the study were found to be increased in NSCLC patients compared to those in healthy controls, with a discrimination power of >90%.[22]

Phillips et al performed two further studies on breath samples from 193 LC patients and 211 controls. In the earlier study, LC was predicted with 84.6% sensitivity and 80.0% specificity by using a model of 16 VOCs. The authors calculated that if applied to a population with 2% prevalence of LC, a screening breath test would have a negative predictive value of 0.985 and a positive predictive value of 0.163.[23] In a later study, the authors used the same database to develop a nonlinear method of multivariate analysis, which accurately identified LC patients using 30 VOCs with 84.5% sensitivity and 81.0% specificity.[24]

Bajtarevic et al used four VOCs that were found in exhaled breath samples of LC patients but not in those of healthy controls to detect LC patients with a sensitivity of 52% and a specificity of 100%. Using 15 and 21 VOCs, they reached a sensitivity of 71% and 80%, respectively, with 100% specificity in both cases.[25] Ligor et al[26] used eight VOCs to detect LC patients with 51% sensitivity and 100% specificity. Fuchs et al examined aldehyde concentrations as biomarkers of LC. Four compounds were found to be significantly higher in LC patients than in smokers and healthy controls.[27] Song et al[28] recognized two VOCs as significantly higher in the breath of LC patients compared with healthy controls. Ulanowska et al[29] reported increased concentrations of 11 VOCs in the breath of 137 LC patients compared with 143 healthy nonsmokers; three VOCs (pentanal, hexanal and nonane) were identified only in the breath of LC patients. Wang et al[30] used 23 VOCs to discriminate LC patients from controls with 96.5% sensitivity and 97.5% specificity.

The GC-MS method was used in even a wider context in a study by Peng et al. In this study, breath samples were collected not only from patients with LC but also from patients with colon, breast and prostate cancers, as well as from healthy controls. According to the results, the system could successfully differentiate between "cancerous" and "healthy" breath and, furthermore, between the breath of patients having different cancer types.[12]

As opposed to all the above-cited studies, in a study comparing LC patients with smokers and healthy controls, Kischkel et al noted that differences in exhalation profiles in cancer and noncancer patients did not persist if physiology and confounding variables were taken into account. The authors concluded that exhaled substance concentrations may depend on a variety of parameters other than the disease under investigation, with the main confounding variables identified as smoking history, inspired substance concentrations, age and sex.[31] In a recent article, Schallschmidt et al reached a similar conclusion when examining 24 VOCs that were suggested as potential cancer markers in previous studies; the authors concluded that the currently available methodology is not able to reliably discriminate cancer patients and healthy controls by using VOCs. Observational studies often tend to note significant differences in the levels of certain oxygenated VOCs, but without the resolution required for practical application, due to low and variable VOC concentrations.[32]

Indeed, the GC-MS method has few major weaknesses. A major one is that it requires preparations and expert personnel

to handle the samples and is also expensive.[33] Another issue is that the method of breath collection affects the concentration of VOCs in the sample. It had been claimed that alveolar breath has the highest levels of exhaled components and the lowest concentrations of contaminants;[34] however, the ability of obtaining alveolar breath samples does not depend only on the collection device itself but also on the collaboration and physical capability of the participant. Finally, another raised question is the stability of compounds and the external effects of different storage bags. Most studies either maintain very short sample storage time to minimize its effect on the breath sample or use tubes that are suitable for long-term storing. Yet, the effects of storage method and equipment are still an issue in allowing a reliable interpretation of the breath analysis.[35–37]

Recognizing the weakness of the method but not abandoning the utility of breath analysis in the detection of LC, Phillips et al[39] showed that breath biomarkers increased the sensitivity, specificity and positive and negative predictive values of chest computed tomography (CT) for LC when the tests were combined in series or parallel.

Nanoarray analysis
eNose

In this method, an exposure of the sensors to a mixture of VOCs changes their electrical resistance. The data are transformed by statistical or structural algorithms to identify various volatile patterns, leading to a production of a "breath print". This method is easy to use and provides highly sensitive results but does not allow quantitative results or identification of unknown substances.[33] As mentioned earlier, different eNose technologies were used in the research for LC detection[40–41]; all provided similar success in the correct recognition of the disease with respect to control groups.

Di Natale et al reported 100% of classification of LC patients versus healthy controls by using eight quartz microbalance (QMB) sensors, and 94% of the reference was correctly classified.[39] Machado et al used a group composed of patients with bronchogenic carcinoma and healthy controls to identify discriminant breath patterns between cancer and noncancer participants; the data were used to create and apply a cancer prediction model prospectively in a separate group of 76 individuals (14 individuals with LC). In the validation study, the eNose had 71.4% sensitivity and 91.9% specificity for detecting LC; positive and negative predictive values were 66.6% and 93.4%, respectively.[40]

Dragonieri et al[42] reported discriminating 85% of NSCLC patients from COPD patients and 90% of NSCLC

patients from healthy controls. McWilliams et al[43] reported distinguishing 25 LC patients from 166 high-risk smokers without cancer with a better than 80% classification accuracy. Gasparri et al examined the breath of 70 LC patients and 76 healthy controls and showed 81% sensitivity and 91% specificity in differentiating the two groups by breath prints. The largest sensitivity was observed for patients with early-stage LC with respect to late-stage disease.[44]

As specified elsewhere in the text, the group of Haick and Peled has reported series of studies using the NaNose system, with high sensitivity and specificity to detect LC as well as other types of cancer. Interestingly, the NaNose was able to discriminate between subtypes of LC (small cell lung cancer [SCLC], adenocarcinoma and squamous cell carcinoma)[45] and mutation status[11] and to track response to therapy and early recurrence.[9]

Colorimetric sensors

This is a slightly different method of nanoarray analysis that uses sensors composed of chemically sensitive compounds that change their colors depending on the chemicals with which they come into contact. Mazzone et al used a prediction model that was developed from breath samples of individuals with LC and other lung diseases (e.g., COPD, sarcoidosis) and healthy controls. Applying the model on another group of participants with a similar disease profile, it was able to predict the presence of LC with 73.3% sensitivity and 72.4% specificity.[46] In a later study, the same authors used the colorimetric sensors to distinguish LC patients from healthy controls with moderate success (*C*-statistic 0.811). Furthermore, individuals with different histologies could be accurately distinguished from one another (*C*-statistic 0.864 for adenocarcinoma versus squamous cell carcinoma).[47]

Potential use of breath analysis as part of screening programs for LC

Screening for LC by LDCT in high-risk populations is becoming more of general practice in the US and Europe. Populations at high risk are considered as individuals aged 55–74 years, who have at least 30 pack-years and who have smoked up to the last 15 years.[5,48] With advancing imaging technology and implantation of screening routines, a significant increase in the number of diagnosed lung lesions can be expected; consequently, more patients will need to go through evaluation of the findings in order to define whether they are malignant and post a threat to patient's life.[49] There is therefore a need for advanced tools that will assist to determine the nature of the detected lesion in as minimally invasive

manner as possible. D'amico et al[51] examined breath samples of 56 patients who were about to go through bronchoscopy due to anomalous chest X-ray. Following the procedure, 28 patients were diagnosed with LC. The use of gas sensor arrays allowed a successful discrimination of 79.3% between cancer and noncancer breath samples. Peled et al analyzed breath samples of 72 patients who were going through investigations for pulmonary nodules by both GC-MS and chemical nanoarrays. A total of 53 of the nodules turned out to be malignant; 19 were benign. The GC-MS analysis identified significantly higher concentration of 1-octene in LC patients' breath. The chemical nanoarrays significantly distinguished benign versus malignant nodules, with 86% ± 4% sensitivity, 96% ± 4% specificity, and 88% ± 3% accuracy.[8]

Potential use of breath analysis to monitor response to anti-cancerous treatment

In a study by Broza et al,[51] breath samples were collected from patients prior to and after lung resection. GC-MS analysis showed that five VOCs were significantly reduced after LC surgery; a nanomaterial-based sensor array distinguished between pre- and postsurgery states. The traced VOCs were attributed to oxidative stress (three VOCs) and to exogenous carcinogens (two VOCs). The study included only a small number of patients and was rather a proof-of-concept study, yet the results indicate that breath pattern changes can be detected not only when LC is present but also after its resection. But what happens in cases when the patient goes though systemic, rather than surgical, treatments? Currently, monitoring response to anti-cancerous treatment in LC is based upon consecutive CT scans. However, time intervals between scans might be too long to allow early identification of treatment failure. Nardi-Agmon et al[9] assessed the use of breath analysis by both GC-MS and nanoarrays in monitoring response to treatment in 39 patients with advanced LC. When one sensor analysis was used, there was 85% success in monitoring disease control. This study suggests that the eNose has also a potential as a quick, simple method to identify lack of response to an anticancer treatment and to prompt treatment reassessment.

Discussion

Diagnosis and evaluation of cancerous disease involved a variety of imaging techniques, depending on the cancer type and available technology. Constant new developments in the imaging field allow earlier detection and better visualization of body tissues. However, the final determination of cancer is made at the tissue level by pathological examination of tissue biopsy taken from a suspected lesion. The process of obtaining a tissue biopsy is not free of complications and bears inconveniences and stress to the patient. There is therefore a need for advanced, minimally invasive diagnosis tools to assist early and reliable recognition of lesions that are suspected to be cancerous.

Currently, no unique tumor marker for LC has been identified. However, further research and development in the field of breath analysis could potentially provide a simple tool to assist early recognition and reliable characterization of suspected lung nodules. To date, the absence of a standardized method for breath analysis among the various researches is a main weakness in assessing the relevance and reliability of the different results obtained. For example, in a review of 10 studies regarding VOCs in LC, 170 different VOCs were detected in total; however, only 17 of them appeared in at least two different studies.[52] In addition, as discussed earlier, independent of the various analysis techniques, the un-unified collection and storage of the breath samples constitute some other major challenges in creating a standardized study method and producing reliable results.[35–37]

A question has risen regarding the difference in breath chemical components between alveolar air and mouth air. While some papers claim that alveolar breath has higher levels of exhaled components,[34,53] in 2015, Capuano et al had conducted a comparative analysis of exhaled breath and air sampled from inside the lungs. The study included 30 patients, 20 with LC and 10 with other lung diseases. The GC-MS and an eNose were used in the analysis of air collected from inside both lungs with a bronchoscopy probe, and the samples were compared with regular breath samples collected from the same patients. The authors found that the diagnostic capability of the eNose did not depend on whether the sampled air was taken from the lung on the side of the tumor itself or from the other lung (in both cases, 90% of correct classification between cancer and noncancer samples was obtained). GC-MS analysis of the air samples from the lung and of breath samples demonstrated a substantial preservation of the pattern of VOCs from inside the lung to the exhaled breath.[54]

The exhaled medium is a feasible approach to support early diagnosis of LC; however, it requires further standardization and clinical implementation. The various results of the GC-MS studies teach us that while it is not possible to identify single specific breath markers for LC, we can reliably say that LC alters the concentrations of a manifold of compounds and modifies the overall chemical composition of the breath. Accepting this important observation brings affront the use of eNose as a useful tool for detecting LC by typical "breath print" rather than by a specific component of breath.

Looking to the future, breath tests are not likely to replace the current imaging modalities and certainly not the need of obtaining actual tissue biopsy to make final and accurate diagnosis (especially as the field of personalized medicine and molecular profiling of tumors is becoming more extensive). However, breath analysis has the potential of constituting a powerful tool to aid and enhance early detection of disease as well as reliable characterization of suspected nodules. Ideally, breath collection and analysis will be done by a simple, easy-to-use tool, which will be available at the doctor's office. Conceptually, breath sample will be collected from a patient with risk factors for cancer development or upon raised clinical suspicion; further on, consecutive breath samples could be collected to assess patient's response to treatment and to detect early signs of recurrence.

Conclusion

LC research is a rapidly developing field. Further understanding of carcinogenesis pathways can potentially provide a better understanding of LC biomarkers, with a more specific research in the breath analysis field. Standardization of breath collection process therefore appears crucial in order to allow further advances in breath analysis research. Breath analysis might not easily replace traditional methods of LC detection and histological characterization, but it surely has a promising potential to augment detection techniques as a noninvasive, non-expensive and rather simple tool.

Disclosure

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References

1. World Health Organization [webpage on the Internet]. *Cancer. Fact Sheet Number 297*. 2015. Available from: http://www.who.int/mediacentre/factsheets/fs297/en/. January 23, 2017.
2. Goldstraw P, Crowley J, Chansky K, et al. The IASLC Lung Cancer Staging Project: proposal for the revision of the TNM stage grouping in the forthcoming (seventh) edition of the TNM classification of malignant tumours. *J Thorac Oncol*. 2007;2(8):706–714.
3. Aberle DR, Adams AM, Berg CD, et al. Reduced lung-cancer mortality with low-dose computed tomographic screening. *N Engl J Med*. 2011;365(5):395–409.
4. Shlomi D, Ben-Avi R, Balmor GR, Onn A, Peled N. Screening for lung cancer: time for large-scale screening by chest computed tomography. *Eur Respir J*. 2014;44(1):217–238.
5. Kauczor HU, Bonomo L, Gaga M, et al. ESR/ERS white paper on lung cancer screening. *Eur Respir J*. 2015;46(1):28–39.
6. Peled N, Ilouze M. Screening for lung cancer: what comes next? *J Clin Oncol*. 2015;33(33):3847–3848.

7. Nakhleh MK, Amal H, Jeries R, et al. Diagnosis and classification of 17 diseases from 1404 subjects via pattern analysis of exhaled molecules. *ACS Nano*. 2017;11(1):112–125.
8. Peled N, Hakim M, Bunn PA Jr, et al. Non-invasive breath analysis of pulmonary nodules. *J Thorac Oncol*. 2012;7(10):1528–1533.
9. Nardi-Agmon I, Abud-Hawa M, Liran O, et al. Exhaled breath analysis for monitoring response to treatment in advanced lung cancer. *J Thorac Oncol*. 2016;11(6):827–837.
10. Hakim M, Broza YY, Barash O, et al. Volatile organic compounds of lung cancer and possible biochemical pathways. *Chem Rev*. 2012;112(11):5949–5966.
11. Peled N, Barash O, Tisch U, et al. Volatile fingerprints of cancer specific genetic mutations. *Nanomedicine*. 2013;9(6):758–766.
12. Peng G, Hakim M, Broza YY, et al. Detection of lung, breast, colorectal, and prostate cancers from exhaled breath using a single array of nanosensors. *Br J Cancer*. 2010;103(4):542–551.
13. Haick H, Broza Y, Mochalski P, Ruzsanyi V, Amann A. Assessment, origin, and implantation of breath volatile cancer markers. *Chem Soc Rev*. 2014;43(5):1423–1449.
14. Feinberg T, Alkoby-Meshulam L, Herbig J, et al. Cancerous glucose metabolism in lung cancer-evidence from exhaled breath analysis. *J Breath Res*. 2016;10(2):026012.
15. Vishinkin R, Haick H. Nanoscale sensor technologies for disease detection via volatolomics. *Small*. 2015;11(46):6142–6164.
16. Krilaviciute A, Heiss JA, Leja M, Kupcinskas J, Haick H, Brenner H. Detection of cancer through exhaled breath: a systemic review. *Oncotarget*. 2015;6(36):38643–38657.
17. van de Kant KD, van der Sande LJ, Jöbsis Q, van Schayck OC, Dompeling E. Clinical use of exhaled volatile organic compounds in pulmonary diseases: a systematic review. *Respir Res*. 2012;13:117.
18. Gordon SM, Szidon JP, Krotoszynski BK, Gibbons RD, O'Neill HJ. Volatile organic compounds in exhaled air from patients with lung cancer. *Clin Chem*. 1985;31(8):1278–1282.
19. Phillips M, Gleeson K, Hughes JM, et al. Volatile organic compounds in breath as markers of lung cancer: a cross-sectional study. *Lancet*. 1999;353(9168):1930–1933.
20. Phillips M, Cataneo RN, Cummin AR, et al. Detection of lung cancer with volatile markers in the breath. *Chest*. 2003;123(6):2115–2123.
21. Poli D, Carbognani P, Corradi M, et al. Exhaled volatile organic compounds in patients with non-small cell lung cancer: cross sectional and nested short-term follow-up study. *Respir Res*. 2005;6:71.
22. Poli D, Goldoni M, Corradi M, et al. Determination of aldehydes in exhaled breath of patients with lung cancer by means of on-fiber-derivatisation SPME-GC/MS. *J Chromatogr B Analyt Technol Biomed Life Sci*. 2010;878(27):2643–2651.
23. Phillips M, Altorki N, Austin JH, et al. Prediction of lung cancer using volatile biomarkers in breath. *Cancer Biomark*. 2007;3(2):95–109.
24. Phillips M, Altorki N, Austin JH, et al. Detection of lung cancer using weighted digital analysis of breath biomarkers. *Clin Chim Acta*. 2008;393(2):76–84.
25. Bajtarevic A, Ager C, Pienz M, et al. Noninvasive detection of lung cancer by analysis of exhaled breath. *BMC Cancer*. 2009;9:348.
26. Ligor M, Ligor T, Bajtarevic A, et al. Determination of volatile organic compounds in exhaled breath of patients with lung cancer using solid phase microextraction and gas chromatography mass spectrometry. *Clin Chem Lab Med*. 2009;47(5):550–560.
27. Fuchs P, Loeseken C, Schubert JK, Miekisch W. Breath gas aldehydes as biomarkers of lung cancer. *Int J Cancer*. 2010;126(11):2663–2670.
28. Song G, Qin T, Liu H, et al. Quantitative breath analysis of volatile organic compounds of lung cancer patients. *Lung Cancer*. 2010;67(2):227–231.
29. Ulanowska A, Kowalkowski T, Trawińska E, Buszewski B. The application of statistical methods using VOCs to identify patients with lung cancer. *J Breath Res*. 2011;5(4):046008.
30. Wang Y, Hu Y, Wang D, et al. The analysis of volatile organic compounds biomarkers for lung cancer in exhaled breath, tissues and cell lines. *Cancer Biomark*. 2012;11(4):129–137.

31. Kischkel S, Miekisch W, Sawacki A, et al. Breath biomarkers for lung cancer detection and assessment of smoking related effects--confounding variables, influence of normalization and statistical algorithms. *Clin Chim Acta*. 2010;411(21–22):1637–1644.

32. Schallschmidt K, Becker R, Jung C, et al. Comparison of volatile organic compounds from lung cancer patients and healthy controls-challenges and limitations of an observational study. *J Breath Res*. 2016;10(4):046007.

33. Saalberg Y, Wolff M. VOC breath biomarkers in lung cancer. *Clin Chim Acta*. 2016;459:5–9.

34. Miekisch W, Schubert JK, Noeldge-Schomburg GF. Diagnostic potential of breath analysis—focus on volatile organic compounds. *Clin Chim Acta*. 2004;347:25–39.

35. Mochalski P, King J, Unterkofler K, Amann A. Stability of selected volatile breath constituents in Tedlar, Kynar and Flexfilm sampling bags. *Analyst*. 2013;138:1405–1428.

36. Beauchamp J, Herbig J, Gutmann R, Hansel A. On the use of Tedlar (R) bags for breath-gas sampling and analysis. *J Breath Res*. 2008;2(4):046001.

37. Mochalski P, Wzorek B, Sliwka I, Amann A. Suitability of different polymer bags for storage of volatile sulphur compounds relevant to breath analysis. *J Chromatogr B Analyt Technol Biomed Life Sci*. 2009;877(3):189–196.

38. Phillips M, Bauer TL, Cataneo RN, et al. Blinded validation of breath biomarkers of lung cancer, a potential ancillary to chest CT screening. *PLoS One*. 2015;10(12):e0142484.

39. Di Natale C, Macagnano A, Martinelli E, et al. Lung cancer identification by the analysis of breath by means of an array of non-selective gas sensors. *Biosens Bioelectron*. 2003;18(10):1209–1218.

40. Machado R, Laskowski D, Deffenderfer O, et al. Detection of lung cancer by sensor array analyses of exhaled breath. *Am J Respir Crit Care Med*. 2005;171:1286–1291.

41. Barash O, Peled N, Hirsch FR, Haick H. Sniffing the unique "odor print" of non-small-cell lung cancer with gold nanoparticles. *Small*. 2009;5(22):2618–2624.

42. Dragonieri S, Annema JT, Schot R, et al. An electronic nose in the discrimination of patients with non-small cell lung cancer and COPD. *Lung Cancer*. 2009;64(2):166–170.

43. McWilliams A, Beigi P, Srinidhi A, Lam S, MacAulay CE. Sex and smoking status effects on the early detection of early lung cancer in high-risk smokers using an electronic nose. *IEEE Trans Biomed Eng*. 2015;62(8):2044–2054.

44. Gasparri R, Santonico M, Valentini C, et al. Volatile signature for the early diagnosis of lung cancer. *J Breath Res*. 2016;10(1):016007.

45. Barash O, Peled N, Tisch U, Bunn PA Jr, Hirsch FR, Haick H. Classification of lung cancer histology by gold nanoparticle sensors. *Nanomedicine*. 2012;8(5):580–589.

46. Mazzone PJ, Hammel J, Dweik R, et al. Diagnosis of lung cancer by the analysis of exhaled breath with a colorimetric sensorarray. *Thorax*. 2007;62(7):565–568.

47. Mazzone PJ, Wang XF, Xu Y, et al. Exhaled breath analysis with a colorimetric sensor array for the identification and characterization of lung cancer. *J Thorac Oncol*. 2012;7(1):137–142.

48. Wood DE, Kazerooni E, Baum SL, et al. Lung cancer screening, version 1. *J Natl Compr Canc Netw*. 2015;13(1):23–34.

49. De Kuning HJ, Meza R, Plevritis SK, et al. Benefits and harms of computed tomography lung cancer screening strategies: a comparative modeling study for the US preventive services task force. *Ann Intern Med*. 2014;160(5):311–320.

50. D'Amico A, Pennazza G, Santonico M, et al. An investigation on electronic nose diagnosis of lung cancer. *Lung Cancer*. 2010;68(2):170–176.

51. Broza YY, Kremer R, Tisch U, et al. A nanomaterial-based breath test for short-term follow-up after lung tumor resection. *Nanomedicine*. 2013;9(1):15–21.

52. Pennazza G, Santonico M, Martinelli E, D'Amico A, Di Natale C. Interpretation of exhaled volatile organic compounds. *Eur Respir Mon*. 2010;49:115–129.

53. van den Velde S, Quirynen M, van Hee P, van Steenberghe D. Differences between alveolar air and mouth air. *Anal Chem*. 2007;79(9):3425–3429.

54. Capuano R, Santonico M, Pennazza G, et al. The lung cancer breath signature: a comparative analysis of exhaled breath and air sampled from inside the lungs. *Sci Rep*. 2015;5:16491.

55. Santonico M, Lucantoni G, Pennazza G, et al. In situ detection of lung cancer volatile fingerprints using bronchoscopic air-sampling. *Lung Cancer*. 2012;77(1):46–50.

56. Hubers AJ, Brinkman P, Boksem RJ, et al. Combined sputum hypermethylation and eNose analysis for lung cancer diagnosis. *J Clin Pathol*. 2014;67(8):707–711.

57. Nisreen S, Cancilla JC, Torrecilla JS, et al. Silicon nanowire sensors enable diagnosis of patients via exhaled breath. *ACS Nano*. 2016;10(7):7047–7057.