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Breathe

The electronic nose: emerging biomarkers in lung cancer diagnostics

Lung cancer is very common and the most common cause of cancer death worldwide. Despite recent progress in the systemic treatment of lung cancer (checkpoint inhibitors and tyrosine kinase inhibitors), each year, >1.5 million people die due to this disease. Most lung cancer patients already have advanced disease at the time of diagnosis. Computed tomography screening of high-risk individuals can detect lung cancer at an earlier stage but at a cost of false-positive findings. Biomarkers could lead towards a reduction of these false-positive findings and earlier lung cancer diagnosis, and have the potential to improve outcomes and treatment monitoring. To date, there is a lack of such biomarkers for lung cancer and other thoracic malignancies, although electronic nose (e-nose)-derived biomarkers are of interest.

E-nose techniques using exhaled breath component measurements can detect lung cancer with a sensitivity ranging from 71% to 96% and specificity from 33 to 100%. In some case series, such results have been validated but this is mostly using internal validation and hence, more work is needed. Furthermore, standardised sampling and analysis methods are lacking, impeding interstudy comparison and clinical implementation. In this narrative review, we provide an overview of the currently available data on E-nose technology for lung cancer detection.

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Key points

- Electronic nose techniques using exhaled breath components measurements have been able to distinguish lung cancer patients from both healthy individuals and patients with nonmalignant respiratory diseases.
- A biomarker for lung cancer could lead to earlier diagnosis and improved treatment monitoring.

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E-nose techniques using exhaled breath component measurements can distinguish lung cancer patients from both healthy individuals and patients with nonmalignant respiratory diseases
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Lung cancer is the most common cause of cancer death worldwide [1]. Despite recent progress in the systemic treatment of lung cancer (checkpoint inhibitors and tyrosine kinase inhibitors), each year, >1.5 million people die because of lung cancer [2]. More than half of the lung cancer patients have advanced disease at the time of initial diagnosis [2]. Key steps to reducing lung cancer-related death are to diagnose lung cancer (and other thoracic malignancies, such as mesothelioma) earlier and to improve the detection of asymptomatic patients. Screening could be key to increasing the chance of cure or prolonged survival [3]. Currently, most screening studies and programmes incorporate computed tomography (CT) scans but at a cost of false-positive findings. The addition of a noninvasively obtained biomarker could provide much needed value to these programmes, both for lung cancer and mesothelioma [3, 4], by improving specificity and reduction of false positives or to provide a more personalised follow-up.

What are biomarkers?

According to the Biomarker Working Group of the US Food and Drug Administration and National Institutes of Health, a biomarker is defined as "a characteristic that is measured as an indicator of normal biological processes, pathogenic processes, or responses to an exposure or intervention, including therapeutic interventions. Molecular, histologic, radiographic, or physiologic characteristics are types of biomarkers. A biomarker is not an assessment of how an individual feels, functions, or survives." Categories of biomarkers [5] include:

- susceptibility/risk biomarkers
- diagnostic biomarkers
- monitoring biomarkers
- prognostic biomarkers
- predictive biomarkers
- pharmacodynamic/response biomarkers
- safety biomarkers

A biomarker should fulfil criteria such as being detectable at a point where it would change the patient pathway or outcome of the disease, or allude to other tests in diagnostic or screening settings.

Why do we need biomarkers in lung cancer?

Diagnostic biomarkers

A biomarker, when available, could be useful in three important roles. First, it could aid in establishing a lung cancer diagnosis, especially as part of a thoracic cancer screening programme with a relatively high percentage of false-positive findings. It could also aid in selecting high-risk patients for a CT screening programme. In lung cancer, no such biomarkers have been validated for clinical use but in other fields, such biomarkers have been of interest (*e.g.* prostate-specific antigen and carcinoembryonic antigen). For lung cancer, the ECLS and bio-MILD trials are currently running to assess blood tumour markers.

Furthermore, the gold standard to diagnose thoracic cancers is a pathology report indicating malignant cells. However, quite often (*e.g.* in severe COPD patients in whom a biopsy cannot be safely obtained), it is not possible to gain enough tissue to establish a diagnosis, and the multidisciplinary team makes decisions based on radiology and clinical details. A diagnostic biomarker would be valuable to help support these teams in their decisions on probability of malignancy.

Predictive biomarkers

The second important role of a biomarker is to predict a future treatment response. The detection of epidermal growth factor receptor (*EGFR*) mutations and *ALK* or other driver mutations are regarded as successful biomarkers in non-small cell lung cancer (NSCLC) [6, 7]. Another biomarker used to predict treatment responses is the programmed death ligand 1 (PD-L1) tumour proportion score. PD-L1 is associated with checkpoint inhibitor efficacy in stage IV, and potentially also in stage III, NSCLC. In clinical trials for mesothelioma, small cell lung cancer and other stages of NSCLC, PD-L1 is also gaining more clinical attention. However, PD-L1 assays are far from perfect, with a low sensitivity and specificity [2]. A significant amount of research activity is aimed at developing new strategies and biomarkers to replace or improve PD-L1. There is a need to improve the sensitivity and specificity of this biomarker, with potential for improvements from new or additional biomarkers.

Biomarkers for monitoring

Thirdly, a biomarker could aid in the serial monitoring of treatment effect, or to distinguish between disease progression and toxicity from treatments such as radiation or immunotherapy. Such a biomarker could be effectively used in parallel to information gained from CT or positron emission tomography scans. In lung cancer, no such biomarkers are available. However, this role for biomarkers is currently being used in other cancer treatments by, for example, prostate-specific antigen and carcinoembryonic antigen.

Ideally, a diagnostic biomarker that is designed to optimise efficacy and safety of low-dose CT screening for lung cancer and subsequent invasive diagnostics should have a high sensitivity and specificity, should be noninvasively obtained, and

should be easy to use and inexpensive. Exhaled breath analysis could fit this profile in the detection of thoracic malignancies. In this narrative review, we provide an overview of the currently available data on electronic nose (e-nose) as a potential diagnostic, predictive or monitoring biomarker for lung cancer treatment.

An e-nose can detect volatile organic compounds (VOC). It is used for both medical and nonmedical purposes. The e-nose for medical purposes is most commonly used to measure VOCs in exhaled breath from patients but has also been used in the assessment of several biological samples including faeces, biopsies, saliva and skin [8–10].

Volatile organic compounds

What are VOCs?

VOCs were identified in the 1970s [11]. Since then, breath analysis has boomed into a high-throughput breathomics research field with >3000 different VOCs discovered in human breath [12, 13]. Most particles in the air are biogenic and emitted through external processes such as the environment and atmospheric pollution [10]. However, due to metabolic processes within the human body, VOCs can be emitted or VOC patterns can be altered [13]. These processes can be physiological but can be also induced by or altered due to disease. Therefore, it is believed that these VOCs cause a specific "smell" or "breathprint" for different diseases. When a concentration of VOCs is measured directly or measured after being captured and stored (*e.g. via* collection bags or canisters), different breathprints or patterns can be detected [14].

VOCs in other diseases

VOC and breathprint detection with e-noses have previously been shown to be effective in respiratory diseases [15]. For COPD, the technique not only allows a COPD diagnosis but can also detect the origin of COPD exacerbations, and can be used in the differential diagnosis of COPD and asthma [15–17]. In asthma, it is used to detect the disease and to determine its phenotype. VOCs are used in cystic fibrosis and in the detection of tuberculosis [18]. Furthermore, nonrespiratory diseases can be detected using an e-nose (*e.g.*Barrett's oesophagus and inflammatory bowel disease) [8, 19]. For other cancer types, positive e-nose studies have been published, including head and neck, bladder, and colon cancer [20].

VOCs in lung cancer

At the foundation of breath pattern research in cancer lies a trial by McCulloch *et al*. [21]. This trial showed that trained dogs were able to detect lung cancer patients in a group of volunteers including healthy subjects. In the 1980s, specific VOCs were demonstrated for the first time in patients with lung cancer [22]. Research then focused on identifying specific VOCs. Different individual VOCs assessed were propranolol, isoprene, acetone, pentane, hexanal and benzene [23]. However, at the time, this proved to be inadequately accurate, expensive and time consuming [24].

As such, interest declined. As technology progressed and superior sensors were developed, interest re-emerged, and new tests were performed on tissues and cell lines in the laboratory [9, 25]. The sensors currently being used focus on pattern recognition. These patterns need to be "learned" first by the machine using artificial intelligence in a manner analogous to the training of dogs used in the original McCulloch study [21]. With this principle, it has now been possible to differentiate lung cancer from healthy subjects and from COPD patients [14, 26–28]. Currently, issues preventing the technique from being widespread in clinical practice include stability of the VOCs, and stability and interchangeability of the devices [29]. If these issues can be resolved, we anticipate that e-noses may find their way to routine practice.

Progress has also been made in mesothelioma. The first tests were published in 2012 [30, 31], showing that molecular pattern recognition of exhaled breath could distinguish mesothelioma patients from healthy controls. More recently, this was confirmed by a study combining breath analysis by gas chromatography–mass spectrometry and an e-nose [32, 33].

E-noses

Different technological principles are used in different e-noses. Differences between e-noses occur at many levels, the most obvious one being the air sampling technique. Some noses require a holding canister while others use air inside a sample balloon. Almost every system needs a contained environment at the moment of sampling and measurement to prevent contamination, especially to reduce the influence of disinfectants or cigarette smoke [24, 34].

Additional differences occur at the level of methodical principles. Some older e-noses measured individual VOCs contrasted by other, more modern noses that assess patterns, the latter requiring training in test sets and validation in an independent set before rendering useful results. Most of the pattern recognition noses are used in combination with artificial intelligence [7].

The most important difference, however, lays in the different type of sensors used inside the technology. The most commonly used techniques are gas chromatography, spectrometry, colorimetry, surface acoustic waves and conductometry [34].

Self-evaluation questions

1. Which of the following statements regarding lung cancer screening is true?
   a. PD-L1 is a suitable biomarker.
   b. Liquid biopsy yields a suitable biomarker.
   c. Electronic nose (e-nose) analysis yields a suitable biomarker.
   d. No suitable biomarker is available.
2. Which of the following statements regarding volatile organic compounds (VOCs) is true?
   a. They can only be sniffed out by dogs.
   b. They can only be sniffed out only by e-noses.
   c. They can be sniffed out by both dogs and e-noses.
   d. They cannot be sniffed out.
3. When was the idea of measuring VOCs to detect lung cancer first published?
   a. In the 1980s
   b. Between 2010 and 2019
   c. Between 2000 and 2010
   d. In this paper
4. In lung cancer patients, the e-nose:
   a. is useless.
   b. can be used to differ between healthy subjects and lung cancer patients.
   c. can replace PD-L1 detection.
   d. can replace computed tomography of the chest.

Gas chromatography

This technique allows the separation of different types of molecules. The air sample is combined with a carrier gas and then moves against a stationary component with a reaction as result. Different substances provide a different responses, with simple chromatography as an obvious example [34, 35].

Spectrometry

Spectrometers are most often combined with gas chromatography. These are used as devices for the identification of specific chemicals. After ionisation of the compounds, the ions from the molecules are separated according to mass-to-charge ratio. This separation normally occurs in a vacuum with a magnetic field. The technique is cumbersome, expensive and difficult to transport, and to date, no point-of-care system with this technique is widely used [34, 36].

Colorimetry

Colorimetric devices work with sensors with chemically responsive dyes. These dyes can be adapted based on the targeted VOC. Multiple dyes can be used in one sensor, allowing patterns of VOCs to be detected [36].

Surface acoustic wave

Acoustic sensors work by exposing their sensors to gases. The gases then change an already emitted acoustic wave due to reactions with the sensor surface. These waves are then analysed for VOC or pattern identification [34].

Conductometry

This technique works with sensors (*e.g.* metal oxide or polymeric sensors) that consist of different metals that allow for various interactions with volatile compounds. Exhaled air is guided over these sensors, allowing redox reactions to occur, resulting in conductivity changes of the sensors [16, 34, 37].

Current level of evidence and current limitations

Diagnostic e-nose biomarkers

When we assess the potential roles of biomarkers in thoracic disease, most VOC-based research has been dedicated to the detection of cancer. To date, e-noses have been able to detect cancer in different settings and have been tested *in vitro*. They can distinguish lung cancer patients from healthy patients, both in volunteers and in those suspected of having cancer [38]. Such studies most commonly assess VOCs emitted by cells in a laboratory setting, with some *in vivo* studies, but mostly in pilot form.

These studies have demonstrated the detection of lung cancer with a sensitivity ranging from 71% to 96% and specificity from 33 to 100% (table 1). Several attempts have been made to validate these results but have mostly only been conducted using internal validation. There is a lack of standardised sampling and analysis method, impeding interstudy comparison and clinical implementation [29, 43, 44].

Predictive e-nose biomarkers

For the role of a biomarker in predicting future treatment responses, research is very limited. In a small pilot study, the e-nose has been able to differentiate between *EGFR*-mutated and wild-type *EGFR* NSCLC; however, more research to assess this is necessary [45]. A recently published study tested an e-nose for the prediction of response to anti-PD-1 therapy in patients with NSCLC with the area under the curve confirmed in the validation set to be 0.85 [46].

Monitoring e-nose biomarkers

For this specific role, at this stage, we have found no published data. However, efforts to study this specific role for the e-nose are being developed.

Table 1 *The most important published studies assessing the efficacy of the e-nose for lung cancer*

First author [ref.]	Year	Lung cancer patients included/total study participants	Type of nose	Result
Philips [36]	2008	95/180	Gas chromatography and mass spectroscopy	Sensitivity: 74%; specificity: 71%
Bajtarevic [35]	2009	220/661	Gas chromatography and mass spectrometry	Sensitivity: 71; specificity: 100%
Dragonieri [39]	2009	10/30	Cyranose	Cross-validation value of 90% correct; sensitivity and specificity not reported
D'Amico [40]	2010	28/148	Gas chromatography and mass spectroscopy	Sensitivity: 85%; specificity: 100%
Gasparri [41]	2016	70/146	Gas sensor array composed of quartz microbalances	Sensitivity: 81%; specificity: 91%
Rocco [12]	2016	23/100	Pneumopipe	Sensitivity: 86%; specificity: 95%
De Vries [15]	2018	35/604	Spironose	Sensitivity 80%; specificity 90%
Huang [28]	2018	56/244	Cyranose	Multiple models Support vector machine Sensitivity: 83%; specificity: 86%
Tirzïte [26]	2018	252/475	Cyranose	Two different models for smokers (sensitivity: 96%; specificity: 92%) and nonsmokers (sensitivity: 96%; specificity: 91%)
Kort [27]	2018	144/290	Aeonose	Multiple models used in the same population Sensitivity: 94%; specificity: 33% (for the NSCLC model)
Van de Goor [42]	2018	52/144	Aeonose	Sensitivity: 83%; specificity: 84%

Summary

E-nose techniques using exhaled breath component measurements can detect lung cancer with a sensitivity ranging from 71% to 96% and specificity from 33 to 100%. However, moment standardised sampling and analysis methods are lacking, impeding interstudy comparison and clinical implementation.

Future research

Future directions of research in this field include the use of artificial intelligence to enhance specificity and sensitivity in lung cancer detection. Large scale validation studies with different devices in different locations according to the advised technical standards are now required to move the field forward [5, 43, 44, 47]. Such new studies could test whether the e-nose could be useful as biomarker for population screenings purposes. A trial with such a design is currently enrolling (www.clinicaltrials.gov identifier NCT02612532), aiming to include 4000 subjects. Other areas for future research include the assessment of the e-nose in prediction of treatment response, treatment monitoring, or the differentiation of treatment complications (*e.g.* pneumonitis) from disease progression [46, 48]. The potential to combine different VOC and radiological or pathological markers to reduce the assumptive risks associated with each one and to enhance their performance is a field that is fertile for future research [49].

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Suggested answers

1. d.
2. c.
3. a.
4. b.

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Conflict of interest

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