Every breath you take: The value of the electronic nose (e-nose) technology in the early detection of lung cancer

Gaetano Rocco, MD, FRCSEd

From the Division of Thoracic Surgery, Thoracic Department, Istituto Nazionale Tumori, IRCCS, Fondazione G. Pascale, Naples, Italy.

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Address for reprints: Gaetano Rocco, MD, FRCSEd, Division of Thoracic Surgery, Thoracic Department, Istituto Nazionale Tumori, IRCCS, Fondazione G. Pascale, Via Mariano Semmola 81, 80131, Naples, Italy (E-mail: g.rocco@istitutotumori.na.it).

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Gaetano Rocco, MD, FRCSEd

Central Message

The e-nose technology can reliably contribute to the determination of a pulmonary nodule's nature, its histologic type, and even its biomolecular features.

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Feature Editor's Note—In this Feature Expert Opinion article, Dr Rocco presents a thoughtfully constructed review detailing the background and significance of an emerging technology in thoracic oncology. Breath samples have recently been found to contain as many as 3000 exhaled volatile organic compounds. These organic compounds are generated through a variety of cellular biochemical processes and are measurable by a number of technologies, well described in this article. On the basis of this premise, exhaled breath fingerprints (volatile organic compound signatures) have proved useful in the differentiation of benign from malignant nodules, which remains a substantial unmet need. As our audience is well aware, lung cancer is the leading cause of cancer-related death in the world, only about 15% of all lung cancer cases are diagnosed as early stage, and the early detection of lung cancer was shown to decrease lung cancer-specific mortality by 20% in the landmark National Lung Screening Trial. Among the abnormal results obtained by low-dose computed tomography screening in this trial, 96.4% were false-positive results, and many of these led to invasive diagnostic procedures. One obvious benefit of a breath print analysis that could discriminate benign and malignant pulmonary nodules is increased accuracy of lung cancer screening and reduced number of unnecessary diagnostic procedures. This is only one of many applications of breath print analyses, however, and this technology has a number of other potential applications in pulmonology, thoracic oncology, and other disciplines, as described in this proceeding. The reader is promised an enjoyable ride through e-nose technology.

Bryan M. Burt, MD

Researchers and clinicians dealing with lung cancer inevitably focus their attention on innovative treatments that can change the fate of our patients. Early diagnosis remains a myth, because lung cancer screening is still plagued by false-positive results and the assessment of the tumor type

requires some form of invasive modality of tumor biopsy, which at times cannot be tolerated because of the patients' often compromised condition.¹⁻³ Liquid biopsy on patients with localized tumors can detect circulating DNA in as many as 55% of plasma samples, but this percentage is bound to increase with tumor stage.^{4,5} As a consequence, promising therapeutic modalities (eg, stereotactic body radiation) are often administered to patients without histologic confirmation, solely on the basis of clinical algorithms predictive of malignancy.⁶ Video-assisted thoracoscopic surgery itself does not rely consistently on preoperative cytohistologic diagnosis, and minimally invasive lung resections can provide both diagnosis and cure at the same time.⁷ Nevertheless, the reported rate of video-assisted thoracoscopic surgery performed for nodules confirmed as benign at final pathology can be as high as 10% to 11%.^{7,8} The wide spectrum of disease stages of lung cancer may suggest different pathways to obtain diagnosis of histotype or to detect tumor or immune system markers for individualized treatment.⁹

Volatile organic compounds (VOCs) are chemical structures generated by cellular metabolism and exchanged from tissue to blood and subsequently with the inhaled air in the alveoli.^{10,11} Strictly speaking, VOCs are markers (signatures) of cellular activity present in the exhaled breath.¹² These compounds can be studied from a quantitative standpoint by using gas chromatography-mass spectrometry, which can provide the exact concentration of

each compound relative to a standard population.^{13,14} Albeit promising, this quantitative breath analysis has not been able to yield a set of lung cancer-specific VOCs, with the possible exception of 4 recently described carbonyl compounds.¹⁵ Currently, the electronic nose (e-nose) assessment technology includes 4 modalities, each with distinct advantages and drawbacks^{16,17} (Figure 1). These modalities, which can be used for qualitative analysis, are infrared spectrometry, gas chromatography–mass spectrometry, solid-state sensors, and mass spectrometry.^{16,17} An example of qualitative analysis of exhalates is the use of gas chromatography–mass spectrometry in a fingerprinting mode.¹³ More recently, a multisensorial platform (BIONOTE) has been proposed, which includes an innovative type of e-nose technology¹⁸ (Figure 2). In this procedure, the exhalate is collected early in the morning from the patient, who is invited to breathe through a device (Pneumopipe, EU patent EP2641537 [A1]2013-09-25) that traps the VOCs onto an absorbent cartridge.¹⁸ The cartridge then undergoes thermal desorption (ie, dissolution at high temperatures) to recapture the VOCs, which are then exposed to gas sensor arrays.¹⁸ In this e-nose modality, gas sensor arrays are composed of quartz crystals microbalance (QCM) with anthocyanin-coated gold electrodes characterized by a baseline oscillation frequency.¹⁹ Once exposed to

the gas sensor arrays, the VOCs induce a mass change on sensors, which translates into a change of their baseline oscillation frequency (ie, sensor activation). Through sensor activation, a pattern of sensor signals—fingerprints^{13,18}—is generated, in a similar fashion to the “combinatorial selectivity” that enables natural olfaction to distinguish multiple different odors.^{13,18} Data analysis and classification between groups of VOCs patterns are performed with a mathematical model that is based on a multivariate test such as partial least square discriminant analysis.^{13,18,20} In 2016, a group of Italian investigators (including me) reported on 100 individuals subjected to lung cancer screening in whom a suspicious lung nodule was identified.¹⁸ These individuals underwent e-nose testing in an effort to differentiate between healthy individuals and those with lung cancer.¹⁸ The results were encouraging, with sensitivity, specificity, positive predictive value, and negative predictive value of 86%, 95%, 83%, and 96%, respectively.¹⁸ Reportedly, irrespective of the sampling technique used in the e-nose technology, exhalate collection and subsequent processing may take as long as 20 minutes, with a reported cost per patient of about €10.¹⁸

The paper by Shlomi and coworkers²¹ on the use of nano-array sensors for breath analysis published in the October 2017 issue of the *Journal of Thoracic Oncology* has the

FIGURE 1. The two main methods of assessment of the volatile organic compounds. The analytic methods aim at identifying the single components by comparing them with known compounds in a reference library, whereas the e-nose technology aims at delineating volatile organic compound pattern recognition algorithms to classify each individual patient profile. *M/Z*, Mass-to-charge ratio; *MS*, mass spectrography; *GC*, gas chromatography. (Reproduced with permission from: van der Schee MP, Paff T, Brinkman P, van Aalderen WMC, Haarman EG, Sterk PJ, et al. Breathomics in lung disease. *Chest*. 2015;147:224-31.)

FIGURE 2. The BIONOTE sensorial platform. After collection of exhaled breath into the Pneumopipe and transfer through a Tenax cartridge (Buchem BV, Apeldoorn, The Netherlands) into the thermal desorption unit, volatile organic compounds are exposed to gas sensor microarrays. The mass alteration induced by the volatile organic compounds will induce a modification of the baseline oscillation frequency in the quartz microbalances (*QCM*), thus generating the breath print, which is then analyzed with partial least squares discriminant analysis (*PLS-DA*), as described in the text. (Modified from: Pennazza G, Santonico M, Scarlata S, Santangelo S, Grasso S, Zompanti A, et al. A non invasive sensor system for the screening of non obstructive sleep apnea syndrome. *Proceedings*. 2017;1:426. Reproduced with permission and under the terms and conditions of Creative Commons Attribution [CC BY] license [<http://creativecommons.org/licenses/by/4.0/>].)

distinct merit of furthering the research in this field. Indeed, this study of 119 patients focused on the possibility for the e-nose technology not only to distinguish between malignant and benign nodules but also to determine its potential endothelial growth factor receptor positivity.²¹ The separation between malignant and benign nodules was done with overall accuracy, positive predictive value, and negative predictive value of 87%, 88%, and 87%, respectively.²¹ In addition, an accuracy of 83%, a sensitivity of 79%, and a specificity of 85% were found when endothelial growth factor receptor positivity was assessed on the basis of specific nanoarray sensor features.²¹

Apart from the use of nanotechnology in manufacturing the gas sensors, the main difference in the e-nose technologies presented by Shlomi and coworkers²¹ compared with the one used by the Italian group¹⁸ resides in the exhalate collection modality (GaSampler polyethylene bags

[QuinTron Instrument Company, Inc, Milwaukee, Wis] vs Pneumopipe), which may not represent a trivial difference because the potential implications for gas preservation and contamination.^{18,21} Nevertheless, the work by Shlomi and coworkers²¹ demonstrates that the use of the e-nose represents today another potentially fruitful application of nanotechnology to thoracic surgery.^{21,22}

The prospective advantages of the introduction into clinical practice of the e-nose technology seem obvious: (1) as a diagnostic tool to verify smoking cessation indirectly in patients enrolled in lung cancer screening programs, since the e-nose can assess fingerprints of chronic obstructive pulmonary disease²³; (2) as a diagnostic tool serving the purpose of identifying high-risk individuals to be subjected to low-dose computed tomographic scanning in the setting of a lung cancer screening program^{15,18}; (3) as a confirming test before scheduling an invasive procedure for a patient

with suspected pulmonary nodule^{18,21}; (4) in the postsurgical follow-up protocols to decide whether and when to proceed to computed tomographic scan or positron emission tomography^{14,18,24}; (5) as a noninvasive method to support the diagnosis of malignancy indicated by clinical algorithms, as is often the case when biopsy is not feasible and the patient needs to be subjected to alternative treatments to surgery, such as stereotactic body radiation^{18,21,25}; and (6) as a noninvasive method to identify lung cancer-related genetic mutations.^{21,26}

There are still, however, limitations to the widespread use of the e-nose that impose caution in the interpretation of the currently available evidence from the literature. There are major hurdles opposing a more diffuse clinical implementation of this technology. The relatively small numbers of the populations subjected to e-nose evaluation and the lack of a standardized and miniaturized device enabling sample collection and data analysis in real time represent the most obvious flaws. In addition, alterations in the composition of the exhaled breath may affect VOC analysis. Examples of such alterations could result from the previous use of drugs, especially chemotherapy agents, and the presence of concurrent viral or bacterial infection.²⁷ In this setting, the ability of e-nose technology to separate lung cancer from chronic obstructive pulmonary disease has been already reported.²⁷ In the future, the possibility of applying the same principles of the e-nose to the assessment of fingerprints in biologic fluids through the so-called e-tongue is being explored.¹⁹ The e-tongue can be used to confirm e-nose and liquid biopsy findings, thus enhancing the overall diagnostic ability in the “no touch” diagnostic lung cancer setting.

In conclusion, the e-nose technology represents a promising, noninvasive modality of obtaining histologic diagnosis of a pulmonary nodule and even of assessing its biomolecular profile. The possible clinical applications of this technology are manifold, but they need to be verified against its current significant limitations.

Conflict of Interest Statement

Authors have nothing to disclose with regard to commercial support.

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