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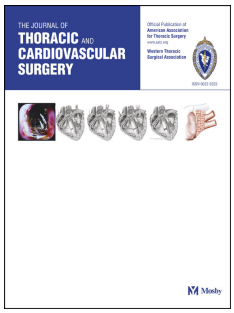

Every breath you take: the value of the electronic nose (e-nose) technology in the early detection of lung cancer

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

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Feature Editor Introduction

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In the accompanying *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 up to 3000 exhaled volatile organic compounds (VOCs). These VOCs are organic compounds generated through a variety of cellular biochemical processes and are measurable by a number of technologies, well described in this article. Based on this premise, exhaled breath fingerprints (VOC signatures) have proven useful in differentiating benign from malignant nodules, which remains a substantial unmet need. As our audience is well familiar, 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 has been shown to decrease lung cancer-specific mortality by 20% in the landmark National Lung Screening Trial (NLST). Among the abnormal results obtained by low dose computed tomography screening in this trial, 96.4% were false positives and many of these lead to invasive diagnostic procedures. One obvious benefit of a breath print analysis that could discriminate benign and malignant pulmonary nodules is to increase the accuracy of lung cancer screening and reduce the 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, described in this proceeding. The reader is promised an enjoyable ride through e-nose technology.

Researchers and clinicians dealing with lung cancer inevitably focus their attention on innovative treatments which can change the fate of our patients. Early diagnosis remains a myth since lung cancer screening is still plagued by false positives and the assessment of the tumor type requires some form of invasive modality of tumor biopsy which at times cannot be tolerated due to the patients' often compromised condition [1-3]. Liquid biopsy on patients with localized tumors can detect circulating DNA in up to 55% of the plasma samples but this percentage is bound to increase with tumor stage [4,5]. As a consequence, promising therapeutic modalities (ie, SBRT) are often administered to patients without histological confirmation based only on clinical algorithms predictive of malignancy [6]. On the other hand, VATS surgery itself does not rely consistently on preoperative cyto-hitological diagnosis; however, minimally invasive lung resections can provide both diagnosis and cure, at the same time [7]. Nevertheless, the reported rate of VATS performed for nodules confirmed benign at final pathology can be as high as 10-11% [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 (ie, 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 (GC–MS) which can provide the exact concentration of each compound compared to 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 four recently described carbonyl compounds [15]. Currently, the e-nose assessment technology includes four modalities, each with distinct advantages and drawbacks [16,17] (Fig. 1). These modalities are infrared spectrometry, gas chromatography mass spectrometry (GC-MS), solid state sensors, and, mass spectrometry and can be used for qualitative analysis [16,17] . An example of qualitative analysis of exhalates is the use of GC–MS in a fingerprinting mode [13]. More recently, a multisensorial

platform (BIONOTE) has been proposed which included an innovative type of e-nose technology [18] (Fig. 2). In fact, 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 absorbing cartridge [18]. The cartridge then undergoes thermal desorption (i.e., dissolution at high temperatures) in order to re-obtain the VOCs that are then exposed to gas sensor arrays [18]. In this e-nose modality, gas sensor arrays are composed of quartz crystals microbalance (QCM) utilizing 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 that translates in a change of their baseline oscillation frequency (ie, sensor activation). Through sensor activation, a pattern of sensor signals – i.e., fingerprints [13,18] – is generated, in a similar fashion to the "combinatorial selectivity" which 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 based on a multivariate test such as the Partial Least Square Discriminant Analysis (PLS-DA) [13,18,20]. In 2016, a group of Italian investigators including the current author 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 and lung cancer affected individuals [18]. The results were encouraging, with sensitivity, specificity, positive and negative predictive values 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 up to 20 minutes with a reported cost per patient of about 10 euros [18].

The paper by Shlomi and coworkers on the use of nanoarray sensors for breath analysis published in the October 2017 issue of the Journal of Thoracic Oncology has the distinct merit to bring the research in this field further ahead [21]. Indeed, this study focused on the possibility for the e-nose technology not only to distinguish between malignant and benign nodules but also to determine its potential EGFR positivity on 119 patients [21]. The separation between malignant and benign nodules

was done with an overall accuracy, positive and negative predictive values 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 EGFR positivity was assessed based on 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 colleagues compared to the one used by the Italian group resides in the exhalate collection modality (ie, GaSampler™ polyethylene bags vs Pneumopipe™) which may not represent a trivial difference given the potential implications on gas preservation and contamination [18,21]. Nevertheless, the work by Shlomi and colleagues demonstrates that the utilization 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: a. As a diagnostic tool to indirectly verify smoking cessation in patients enrolled in lung cancer screening programs since the e-nose can assess fingerprints of COPD [23]; b. As a diagnostic tool serving the purpose of identifying high risk individuals to be subjected to low dose CT scanning in the setting of a lung cancer screening program [15,18]; c. As a confirming test prior to scheduling an invasive procedure for a patient with suspected pulmonary nodule [18,21]; d. In the post-surgical follow-up protocols to decide if and when to proceed to CT scan/PET [14,18,24]; e. As a non-invasive method to support the diagnosis of malignancy indicated by clinical algorithms. This is often the case when biopsy is not feasible and the patient needs to be subjected to alternative treatments to surgery, ie, SBRT [18,21,25]; f. As a non-invasive method to identify lung cancer-related genetic mutations [21,26].

However, there are still limitations to the widepread 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 numerosity 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 ones. Also, alterations in the composition of the exhaled breath may affect VOCs analysis. Examples of such alterations could result from the previous use of drugs, expecially 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 COPD 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, non invasive modality of obtaining histological diagnosis of a pulmonary nodule as well as assessing its biomolecular profile. The possible clinical applications of this technology are manifold but they need to be verified against its current significant limitations.

Legends

Fig. 1. The two main methods of assessment of the VOCs. The analytic methods aim at identifying the single components comparing them with known compounds in a reference library whereas the e-nose technology tends at delineating VOCs pattern recognition algorithms in order to classify each individual patient. Reproduced with permission from van der Schee MP et al. Breathomics in Lung Disease. Chest 2015;147:224-31

Fig. 2. The BIONOTE sensorial platform. After collection of exhaled breath into the Pneumopipe and transfer through a Tenax cartidge into the thermal desorption unit, VOCs are exposed to gas sensor microarrays. The mass alteration induced by the VOCs will induce a modification of the baseline oscillation frequency in the QCM thus generating the breathprint which is then analyzed with PDA (See text). Modified from Pennazza G, et al. "A non invasive sensor system for the screening of non obstructive sleep apnea syndrome" Proceedings, 2017; 1:426; doi:10:3390/proceedings1040426 – www.mdpi.com/journal/proceedings. Reproduced with permission and under the terms and contiions of Creative Commons Attribution (CC BY) license (http://creativecommons.org/licenses/by/4.0/).

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