Review

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The Electronic Nose in Respiratory Medicine

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Electronic nose · Chemical sensors · Volatile organic compounds · Breathomics · Airway inflammation · Asthma · Chronic obstructive pulmonary disease · Lung cancer

Abstract
Several volatile organic compounds have been identified in exhaled breath in healthy subjects and patients with respiratory diseases by gas chromatography/mass spectrometry. Identification of selective patterns of volatile organic compounds in exhaled breath could be used as a biomarker of inflammatory lung diseases. An electronic nose (e-nose) is an artificial sensor system that generally consists of an array of chemical sensors for detection of volatile organic compound profiles (breathprints) and an algorithm for pattern recognition. E-noses are handheld, portable devices that provide immediate results. E-noses discriminate between patients with respiratory disease, including asthma, COPD and lung cancer, and healthy control subjects, and also among patients with different respiratory diseases. E-nose breathprints are associated with airway inflammation activity. In combination with other 'omics' platforms, e-nose technology might contribute to the identification of new surrogate markers of pulmonary inflammation and subphenotypes of patients with respiratory diseases, provide a molecular basis to a personalized pharmacological treatment, and facilitate the development of new drugs.

Introduction

Several volatile organic compounds (VOCs), including isoprene, 1,2-pentadiene, acetone, ethanol, pentane and ethane, have been identified in the exhaled breath of healthy subjects and patients with respiratory diseases by gas chromatography/mass spectrometry (GC/MS) [1, 2]. Identification of selective VOC patterns in exhaled breath could be used as a biomarker of lung diseases [3–8]. An electronic nose (e-nose) is an artificial sensor system which enables a qualitative and/or quantitative description of volatile mixtures [9–11]. The e-nose working principle, which is based on the human olfactory system, involves three steps: (1) binding of volatile substances to a sensor array; (2) generation of sensor changes which result in unique patterns of signals, and (3) integration of signal patterns for classification purposes [10]. However, there are important differences between the human olfactory system and artificial noses. These differences are

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mainly related to sensor system complexity, sensitivity, specificity, types of sensor (receptors vs. chemical sensors), characteristics of volatiles detected and effects of ambient factors (e.g., humidity) on volatile identification. The human olfactory system is much more complex than artificial noses as nasal epithelium contains between 10 and 100 million receptors which bind odorant volatile molecules even at the parts per trillion (ppt) level with high specificity [12, 13]. E-noses are able to detect non-odorant gases (e.g., carbon monoxide), but are not sensitive to several substances recognized by the human nose, can be affected by background substances such as water vapour, and are currently much less specific and sensitive [9–14]. E-noses generally consist of an array of chemical sensors [9–14]. However, technologies, including optical sensor systems, MS, ion mobility spectrometry, selected ion flow tube mass spectrometry (SIFT-MS), GC and infrared spectroscopy are also referred to as e-noses [9–14]. The classical e-nose, based on chemical sensor technology, is sensitive to the totality of the compounds rather than to a specific one [9, 10, 14]. Individual chemical sensors are globally selective as each sensor detects more chemicals and each chemical is detected by more than one sensor. Due to poor specificity to individual volatiles, chemical sensor arrays are not generally suitable for identifying single gases/volatiles in complex mixtures such as human breath, for which different e-nose techniques including SIFT-MS, thermal desorption GC/MS and GC/MS coupled with solid phase extraction or micro-extraction are required [15]. The steady-state frequency, resistance, potential or current shifts of the e-nose sensors produce a pattern ('breathprint'), and a collection of measurements produces a set of breathprints that are analysed by a pattern recognition algorithm for classification purposes [16]. Acoustic e-noses detect changes in the propagation (velocity and/or amplitude) of acoustic waves through or on the surface of the sensor coating material due to sorption of volatile compounds [16]. In the optical sensor systems, changes in light properties or characteristics including absorbance, fluorescence, optical layer thickness and polarization are measured [9, 16]. Calorimetric e-noses are made from pellistors ('pelletised sensors') and detect temperature or heat change derived from chemical reactions between catalytic beads and ambient gases. This leads to an increase in the sensor resistance that generates a signal [16].

The e-nose is potentially useful for discriminating between asthmatic patients and healthy subjects [3, 17], between patients with asthma of different severity [17], between patients with lung cancer and healthy subjects and/or patients with non-cancer lung disease [5, 6, 18–21], between patients with lung cancer and COPD [18] and between patients with asthma and COPD [4, 22]. Once chemical sensor arrays have shown between-group differences based upon breath VOC profiles, GC/MS can be used for identifying those VOCs responsible for discriminating and quantifying their concentrations. This approach might provide new pathophysiological insights, identify novel biomarkers of pulmonary disease, and have implications for diagnosis and therapeutic monitoring of patients with respiratory disease. In this review, the methodology of e-nose and its potential applications to respiratory medicine are discussed.

E-Nose Methodology

The e-nose methodology is relatively new. Different internal laboratory standardized procedures for collection of exhaled breath and e-nose analysis are available, but none is globally accepted.

Collection of Exhaled Breath

Issues related to exhaled breath sampling regard the type of sampling (total vs. alveolar breath), the effect of ambient substances, the duration of sampling (single breath vs. fixed-time breathing) [15], the effect of expiratory flow [17], type of collecting bags, effect of humidity and VOC recovery.

Total versus Alveolar Breath Sampling

There are two main techniques for collecting exhaled breath: mixed expiratory sampling in which total breath including dead space air is collected, and alveolar sampling in which alveolar breath is collected [3, 15, 23] (fig. 1). Mixed expiratory sampling is easy to perform, but is affected by dead space air dilution [15]. Alveolar breath sampling requires additional equipment, but reduces contaminant concentrations [15, 23].

In one protocol [4, 17, 18, 22], an equilibration phase (wash-in) with VOC-filtered room air is performed before breath sampling to reduce the interference of ambient VOCs [17]. Subjects, while wearing a nose-clip, are asked to breathe tidally VOC-filtered air for 5 min into a 2-way T-shape non-rebreathing valve with an inspiratory VOC filter and an expiratory silica reservoir to reduce sample water vapour, which can affect sensor response [9, 16]. Then, subjects are asked to inhale to total lung capacity and perform a forced vital capacity manoeuvre into a

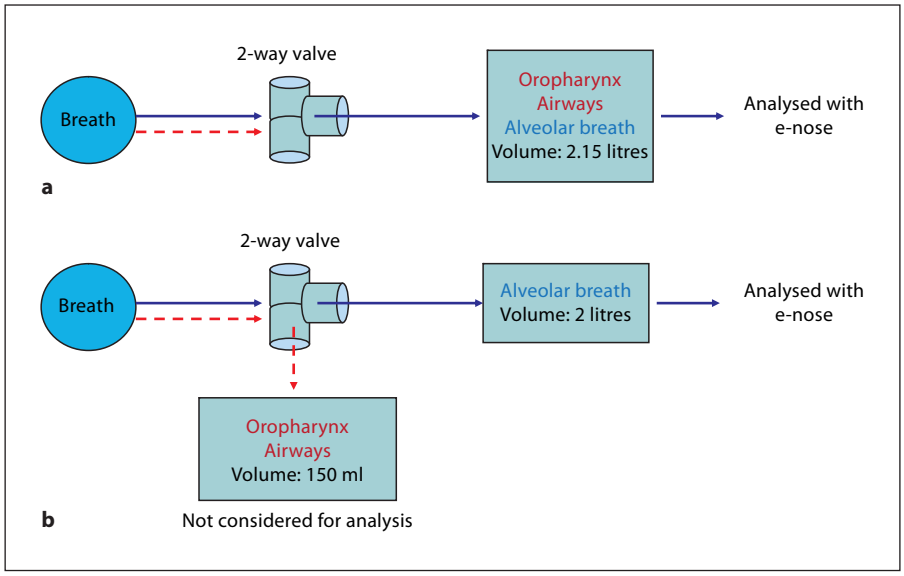

Fig. 1. Breath sampling including two procedures for collecting total exhaled breath (**a**) and alveolar breath (**b**).

Tedlar bag against an expiratory resistance of 20 cm H_2O to close the soft palate and obtain an expiratory flow of 0.1–0.2 litres/s [4, 17, 18, 22]. This breath sampling procedure minimizes the effect of ambient VOCs on e-nose analysis, but does not enable comparison of breath VOC profiles from different lung compartments as total exhaled breath is collected.

Another protocol includes two procedures for collecting exhaled breath for studying the differences between total exhaled breath and alveolar breath (fig. 1) [3]. In the first sampling procedure, subjects are asked to inhale to total lung capacity and to fully exhale into a mouthpiece connected to a Tedlar bag through a 2-way T-shape nonrebreathing valve (fig. 1a) and total exhaled breath is collected. In the second sampling procedure, subjects are asked to repeat the manoeuvre. The first 150 ml, considered as dead space volume, are collected into a separate Tedlar bag and discarded, and the remaining breath volume, principally derived from the alveolar compartment, is analysed (fig. 1b) [3]. This approach enables differential breath sampling, but requires adjustment for ambient VOCs. A protocol combining a wash-in phase with VOC-filtered air and differential breath sampling has been proposed [21]. In another study, subjects performed tidal breathing of room air for 12 min while exhaling into a device designed to draw their breath across a colorimetric sensor array [19].

The possibility of breath sampling directly into sorbent traps (e.g., Tenax TA and Tenax GR) is under investigation. This approach has the advantage of increasing sample breath VOC concentrations and stability, but is currently limited by technical issues (e.g., high resistance during breath sampling).

Effect of Ambient Substances

To correct for the effect of ambient volatiles on exhaled breath two approaches are possible: calculating alveolar gradients [1, 15] or having subjects breathe gas/volatile-free air for a certain time before measurement (wash-in) [17]. Alveolar gradients are obtained by subtracting inspiratory from expiratory volatile concentrations [2, 15]. Wash-in with volatile-free air can be more effective but less feasible in the clinical setting [1, 15]. However, calculating alveolar gradients is affected by the complexity of pulmonary kinetics of volatiles that, in turn, depend on breathing patterns, ventilation perfusion ratio in the lung and alveolar concentration gradients of volatile compounds [15]. Moreover, it should be considered that pulmonary kinetics is affected by lung disease and that different respiratory diseases can have different effects on pulmonary kinetics.

Single Breath versus Fixed-Time Breathing

Some authors consider breath analysis in samples collected during a certain time preferable over single-breath analysis, as breath-to-breath volatile concentrations may vary considerably and averaging is often necessary [15]. However, other groups have obtained good classification rates using the single-breath method [3–6, 17].

Effect of Expiratory Flow

Expiratory flow rate and ambient temperature for breath sampling have to be standardized [9]. In single-breath analysis, the possible effect of expiratory flow during vital capacity sampling was studied in healthy non-smokers by using 100–200 ml/s and 300–500 ml/s in a random order with a 30-min interval [17]. Different expiratory flow rates were achieved by changing expiratory resistance (20 cm H_2O to obtain an expiratory flow between 100 and 200 ml/s) during the vital capacity manoeuvre [17]. Within this flow range, expiratory flow has a limited effect on e-nose measurements [17]. For this reason, some authors perform single-breath sampling at an expiratory flow between 100 and 200 ml/s [17]. However, further studies are required to formally address this issue. The effect of expiratory flow on e-nose measurements should particularly be studied at different expiratory flow ranges, after breathing for a certain time (as opposed to single-breath sampling), and in patients with respiratory disease.

Type of Collecting Bags

Plastics bags coated with inert materials including polyvinyl fluoride (Tedlar®) [3, 4, 17, 18, 22, 24], fluorinated ethylene propylene copolymer (Teflon®) [25] or polyethylene terephthalate (Mylar®, Nalophan®) [5, 26], or electropolished stainless steel canisters can be used for collecting breath samples [15]. Tedlar bags, which are recommended by the US Environmental Protection Agency for ambient gas sampling [27], are widely used for breath sampling from patients with respiratory disease [3, 4, 17, 18, 22, 24]. They are manufactured from a polyvinyl fluoride film which is chemically inert and does not react with or alter the composition of a wide range of breath volatiles. However, polar and reactive compounds like methyl ethyl ketone, formaldehyde, methanol, 1-butene and acetone can adhere to Tedlar film, making their collection less efficient [27]. Particularly, if bags are exposed to direct sunlight, Tedlar materials can release hydrocarbons [27], N,N-dimethylacetamide and phenol [24, 28]. To reduce hydrocarbon concentrations, Tedlar bags should be purged with ultrapure nitrogen and direct exposure to sunlight should be avoided [27]. GC/MS analysis after thermal desorption of volatiles from fibres exposed to empty Tedlar bags should be undertaken to establish that contaminant levels are acceptable [27]. Although N,N-dimethylacetamide and phenol can pollute the Tedlar bag content, these compounds are unlikely to interfere with the analysis of breath VOCs [24]. Stability of a selected panel of standard VOCs (benzene, toluene, p-xylene, styrene, methyl ethyl ketone, methyl isobutyl ketone, butyl acetate and isobutyl alcohol) in Tedlar bags is acceptable up to 2 days from air sample collection [29]. Similar results were reported when human breath samples were analysed [24]. Decreases from VOCs in breath samples during storage are time dependent, but limited to less than 10% up to 52 h [24]. To increase reproducibility, a fixed point in time after sample collection should be chosen for breath analysis [24]. Variations in decreases in breath VOCs over time are generally smaller than inter-individual differences for most breath VOCs [24].

Effect of Humidity

Sample humidity seems to have no effect of VOC stability in the Tedlar bags [24], whereas it can affect the behaviour of some types of chemical sensor arrays including those based on conduction polymer sensors or metal oxide sensors [9, 16, 30]. Likewise, ambient water vapour concentrations, ranging from <300 ppm to >73,000 ppm, and particularly their changes on a daily basis, make the accurate detection of volatiles at sub-ppm concentrations very difficult [31]. Possible solutions to this problem include the use of silica gel to reduce breath humidity during sampling [4], heating of samples, maintaining the sensor chamber at a fixed temperature [16] and the choice of types of sensors less sensitive to water vapour (e.g., colorimetric sensor arrays) [31].

VOC Recovery

Relative recoveries of VOCs depend on the physical-chemical properties of the single VOC and the bag materials [29]. For Tedlar bags, recovery was greater for the lower molecular weight aromatic VOCs than for the high molecular weight aromatics, whereas non-aromatic VOCs show the opposite behaviour [29]. The use of polyester aluminium bags as a replacement for Tedlar bags is likely to yield more reliable data in the analysis of VOCs in terms of greater stability and sensitivities, at least over periods of less than 3 days [29]. The suitability of different polymer bags (Nalophan, Tedlar, Teflon and FlexFoil) for storage of breath volatile sulphur compounds has been formally addressed [32]. FlexFoil bags seem to have the best performance for volatile sulphur compound storage up to 24 h in terms of recovery, background, light sensitivity, reusability and matrix effects [32]. Alternatively, Tedlar bags can be used for storing times up to 6–8 h [32]. In analogy to the volatile sulphur compound study, comparative studies aiming at assessing the performance of different types of collecting materials for breath VOC

with different physical-chemical characteristics should be undertaken. Teflon-coated bags, which are inert to virtually all chemicals and have been reported to have the lowest amounts of chemical impurities [28], and Mylar-coated bags have been used for breath sampling in patients with respiratory disease [5, 25] and for headspace sampling from microbiological cultures [33]. For volatile fatty acids, solid-phase micro-extraction fibres have the highest mean recovery followed by sorbent 2,6-diphenylene-oxide polymer resin (Tenax TA) sorbent tubes, polyethylene bags, Teflon bags and Tedlar bags [28]. A critical review of the different types of sampling devices for e-nose analysis is difficult due to the lack of head-to-head studies aiming at comparing the behaviour of the different collecting bag materials exposed to human breath. These studies are required for optimization and standardization of the e-nose technique. Likewise, formal GC/MS studies aimed at identifying and quantifying volatiles possibly released from the different coating materials and their potential effects on breath analysis performed with chemical sensor arrays are warranted. As many breath VOC concentrations are in the nano- to picomolar (ppbv to pptv) range [34], preconcentration of breath samples obtained with adsorption onto sorbent traps or coated fibres (solid phase micro-extraction) can be required [15]. Different adsorbent materials, including organic polymers (e.g., Tenax), activated charcoal, carbon molecular sieves and graphitized carbon, are used in sorbent traps [15]. Different adsorbents can also be combined in a single sorbent trap [15] to improve the quality of preconcentration. The use of sorbent traps has also the advantage of improving breath sample stability.

E-Nose Analysis: Principles and Devices

The setup for e-nose analysis generally consists of an e-nose with software for data analysis, a collecting bag containing VOC-filtered ambient air for baseline and a collecting bag containing the breath sample. However, not all techniques require or use sample collecting bags, baseline bags and on-board analysis software [1, 9, 15].

A list of commercially available e-noses, not including the recent NA-NOSE, is available [9]. Some e-noses based on chemical sensor arrays are currently prototypes, not yet available on the market [3, 6, 20, 21, 35–38]. Sensor arrays are the most common approach to e-nose technology due to their large-scale use and similarity to biological sensors. Different types of sensors, including metal-oxide semiconducting gas sensors, conducting polymer gas sensors, electrochemical gas sensors, catalytic field-effect sensors (metal-oxide semiconductor field effect transistors), acoustic sensors (quartz crystal microbalance sensors, surface and bulk acoustic wave gas sensors), optical gas sensors, calorimetric sensors, infrared sensors, fluorescence sensors and colorimetric sensors, as well as sensing materials, are available [16]. Advantages and limitations associated with individual e-nose sensor types include specificity, response and recovery times, range of volatiles detected, sensitivities, operating temperatures, physical size, temperature and humidity effect on sensor functioning, portability, cost and circuitry complexity [16]. Metal oxides semiconducting sensors have high sensitivity but poor precision, require high operating temperatures and are sensitive to humidity [16]. Conducting polymer sensors are sensitive to many VOC, have a short response time and operate at ambient temperature, but are sensitive to humidity and temperature, and sensor life is limited [16]. Quartz crystal microbalance sensors have good precision and high sensitivity, but have poor signal-to-noise ratio and are sensitive to humidity and temperature [16]. Surface acoustic wave sensors have high sensitivity, good response times and are sensitive to virtually all volatiles, but are temperature sensitive, their electronics is complex and specificity to chemical groups is affected by sensor coating materials [16]. In optical sensor systems, the most direct method involves measuring the gas/volatile absorbance in a specific frequency range, but they lack sufficient sensitivity for compounds in the lower concentration range as is the case of many breath VOCs [9]. Calorimetric sensors have a fast response and recovery time and high specificity for oxidized compounds, but require high temperature operation and are only sensitive to oxygen-containing volatiles [16]. Using a carbon black polymer-based gas sensor array, limits of detection values, determined for breath volatiles including acetic acid, toluene, ethanol, acetone, *n*-pentane and isoprene, were in the range of 80–240 ppb [39]. Using an array of quartz microbalance sensors covered with molecular films of different metalloporphyrins, under controlled laboratory conditions, calibration curves for volatiles with different hydrophilia and polarity such as ethanol, hexane and ethyl acetate have shown experimental limits of detection of 65, 141 and 171 ppb, respectively [40]. These detection limit values can still be too high for some breath VOC which are detected in the lower ppb range. Improving the detection limit of e-noses based on chemical sensor arrays is one of the priorities in this research field. Increasing the sensor array number does not necessarily improve the e-nose classification performance as that could amplify the noise and/or generate redundancy of information [9, 11]. At present, the ideal sensor array does not exist. Selecting

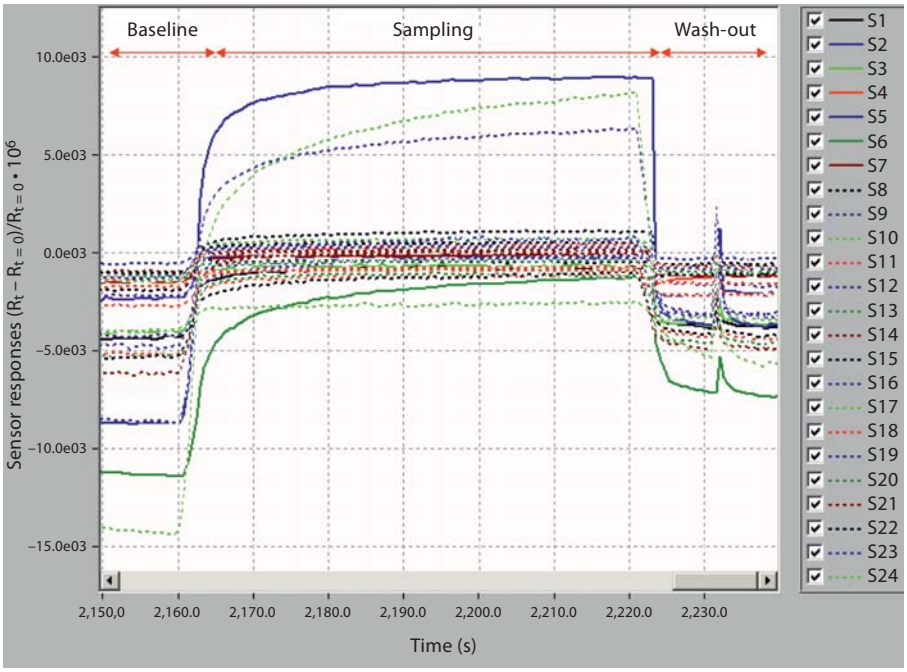

Fig. 2. Typical sensor response (Rmax – R0/R0) obtained with an e-nose. Each line represents a sensor response in terms of resistance (R) changes over baseline values.

a sensor array based upon its application and chemical characteristics of volatiles to be detected might be a rationale strategy for choosing the 'right' e-nose [9, 11, 16].

The Cyranose 320®, an e-nose currently produced by Intelligent Optical Systems Inc., Baldwin Park, Calif., USA, consists of an array of 32 chemical sensors made from composites of an inorganic conductor (carbon black) and insulating organic polymers [41]. The measurement is based on a resistance variation in each chemical sensor when exposed to a VOC mixture (fig. 2). The differential responses across the array (resistance shifts) are composed in patterns and analysed by pattern recognition algorithms [42].

A prototype of e-nose, LibraNose (University of Tor Vergata, Rome, Italy) [3, 6], contains an array of eight quartz microbalance gas sensors coated by molecular films of metalloporphyrins [43]. Sensors detect the concentrations of chemicals absorbed in the sensitive films through the changes of resonant frequency that is proportional to the absorbed mass [3].

Another device, the MOSES II eNose® (GSG Mess- und Analysengeräte GmbH, Bruchsal, Germany), contains eight metal-oxide sensors and eight quartz microbalance sensors, two very different sensor technologies that provide complementary information on the adsorbed volatile compounds [38]. The Nanoscale Artificial Nose (NA-NOSE), at present non-commercially available, consists of five sensors that are based on spherical gold nanoparticles [21].

A colorimetric sensor array consists of 36 spots composed of different chemically sensitive compounds (e.g., metalloporphyrins) impregnated on a disposable cartridge [19]. The colour spots change depending on the VOCs bound [19, 44]. Colour changes are converted in numerical vectors [19].

Fraction of exhaled nitric oxide (F_ENO) analysers, which provide immediate results and are used for assessing airway inflammation in patients with asthma [45], can also be considered as e-noses [9]. F_ENO analysers, which have been approved in the clinical setting [46, www.aerocrine.com], include a portable, hand-held device, NIOX MINO® (Aerocrine, Solna, Sweden), based on a relatively selective chemical sensor [47]. F_ENO analysers will not be discussed further in this article.

Hybrid systems have the advantage of combining the high sensitivity of classical e-noses based on chemical sensor arrays with the high specificity of different e-nose techniques including GC/MS [9]. The e-nose Prometheus® (Alpha MOS, Toulouse, France) is an example of a hybrid system which combines a sensor array with a fingerprint mass spectrometer [9, 16]. The sensor array consists of 18 metal oxide sensors arranged in three chambers each containing six sensors [9]. The fingerprint mass spectrometer consists of a quadruple mass filter and

an electron impact ionizer. The Z-Nose® (Electronic Sensor Technology, Newbury Park, Calif., USA), which is available in a portable version, combines a surface acoustic wave detector with GC (www.estcal.com).

Chemical Sensor Array Data Analysis: Algorithms for Pattern Recognition

The analysis of breath VOC data obtained with chemical sensor arrays requires multivariate statistical algorithms [48]. The field of pattern recognition has general applications in systems biology which are far beyond e-nose data including biomedical magnetic resonance [49] and 'omics' technologies such as metabolomics, proteomics, transcriptomics, genomics and lipidomics. There are two types of pattern recognition: exploratory techniques, which are generally unsupervised, and confirmatory techniques, which are supervised [50]. Pattern recognition analysis of data obtained with an e-nose can be performed on-board or off-line. On-board data analysis can include cross-validation, principal component analysis (PCA) and canonical discriminant analysis. The inbuilt software calculates the cross-validation value, a cross-validation estimate of error. Cross-validation is an internal tool to check the training set and the model that has been built. The ability to discriminate between groups can be quantified by the Mahalanobis distance, which expresses the distance between group means in units of standard deviations [51]. PCA, used as a tool in exploratory data analysis and for making predictive models, transforms a number of possibly correlated variables into a smaller number of uncorrelated variables called principal components [48]. Canonical discriminant analysis is a method to find a linear combination of features that characterize or separate two or more classes [48]. Off-line data analysis with different classifiers include partial least squares-discriminant analysis [6, 20], support vector machines (SVMs) [5, 52, 53], random forest technique [19] and multi-layer neural networks [3, 54]. There are many different methods of e-nose data analysis, but at present there is no consensus on the best method. The choice of the pattern recognition algorithm should be based on the type of data (linear vs. non-linear) and e-nose application. Choosing a classifier which is suitable for the sensor response patterns that will be generated is essential for achieving the best e-nose classification performance [50].

Repeatability and Reproducibility of E-Nose Measurements

At present, available data on repeatability and reproducibility of e-nose measurements are derived from one study in which Cyranose 320 was used [4]. Sensor responses from two consecutive breath samples were assessed for comparing within-day repeatability. Intraclass correlation coefficients ranging from 0.65 to 0.91 (mean 0.80) indicate similar sensor responses [4]. Data on between-day repeatability, assessed in 18 healthy subjects, indicate that sensor responses, analysed by paired t testing, are similar [4]. Technical reproducibility, assessed by analysing the same breath samples with two identical e-noses, was similar [4]. More studies are required to assess the repeatability of Cyranose 320 measurements in different centres. Likewise, repeatability studies for other types of e-noses are warranted.

Applications of E-Nose in Respiratory Medicine

E-nose technology has been used for analysing the breath patterns of patients with asthma [3, 4, 17, 22], COPD [4, 18, 55], lung cancer [5, 6, 18, 20, 21], cystic fibrosis [56–58], healthy smokers [4] and healthy non-smokers [3–5, 17, 22].

Asthma

Using a chemical sensor array, breath VOC patterns were analysed in 10 steroid-naïve non-smoking atopic patients with mild asthma, 10 non-smoking atopic patients with severe asthma, 10 healthy non-smokers aged 18–45 years and 10 healthy non-smokers aged 46–70 years [17]. Breath samples were collected after an equilibration phase with VOC-filtered room air (wash-in). Data were analysed by on-board software and, then, offline confirmatory analysis was performed by double cross-validation implementation of linear discriminant analysis of principal component reduction [59]. The e-nose discriminated between patients with mild asthma and healthy subjects aged 18–45 years (cross-validation value: 100%; Mahalanobis distance: 5.32) and between patients with severe asthma and healthy subjects aged 46–70 years (cross-validation value: 90%; Mahalanobis distance: 2.77), whereas the e-nose discrimination ability between patients with mild and severe asthma was lower (cross-validation value: 65%; Mahalanobis distance: 1.23) [17]. No discrimination between healthy control groups was observed (cross validation value: 50%; Mahalanobis distance: 1.56) [17].

In patients with a physician-based diagnosis of asthma, the diagnostic performance of a prototype of an e-nose (LibraNose) was compared with pulmonary function tests and F_ENO [3]. Twenty-seven steroid-naïve non-

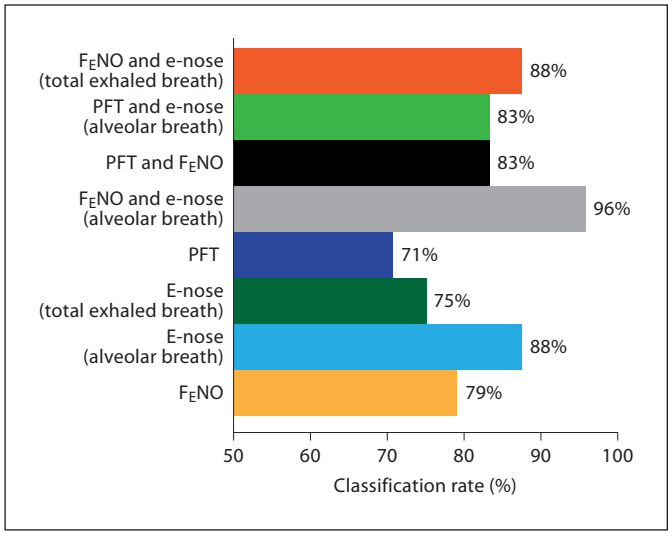

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Fig. 3. Diagnostic performance of an e-nose, F_ENO and pulmonary function testing (PFT) in patients with intermittent and mild persistent asthma [data from 3]. The diagnostic performance was determined in terms of the number of correct identifications of asthma diagnosis in the testing dataset and was related to the best performances obtained with a neural network classification model for each specific case.

smoking atopic patients with intermittent and mild persistent asthma and 24 healthy non-smokers were included in this study. Two procedures for collecting exhaled breath were followed to study the differences between total and alveolar air. A subgroup of 7 patients with asthma and 7 healthy subjects participated in a study with MS fingerprinting [60] as an independent technique for assessing between group discrimination and short-term stability of breath samples [3]. PCA and feed-forward neural network were used to classify e-nose, F_ENO and spirometry data. To validate the model, the total datasets were divided into training and testing sets. To test the presence of any sensor drift, the first data collected were used for training and the remaining for testing [3]. The diagnostic performance was determined with the test datasets in terms of the number of correct identifications of asthma diagnosis based on National Asthma Education and Prevention Programme guidelines [61]. The best results were obtained when the e-nose analysis was performed on alveolar air in which the highest concentrations of endogenous VOCs had been reported [23]. Diagnostic performance for e-nose alone, F_ENO alone and pulmonary function testing alone was 88%, 79% and 71%, respectively. The combination of e-nose and F_ENO had the highest diagnostic performance for asthma (96%; fig. 3) [3]. MS fingerprints of VOCs confirmed the validity of the e-nose-based classification and demonstrated that breath samples are stable for at least 48 h [3]. The relatively small number of subjects included in this study precludes definitive conclusions on asthma diagnostic performance of e-nose alone or in combination with other methods for which large powered studies are warranted. The utility of e-nose technology as a diagnostic tool for screening of patients with asthma has to be established in large prospective studies.

Twenty-one asthma patients with fixed airway obstruction (fixed asthma), 39 asthma patients with reversible airway obstruction (classic asthma) and 40 patients with COPD (GOLD stages II–III) were included in a cross-sectional study [22]. Breath samples were collected after a 5-min equilibration phase with VOC-filtered air and analysed [22]. External validity in newly recruited patients (validation set) was tested with a previous and independent training set [22]. PCA, canonical discriminant analysis and area under the curve (AUC) of receiver operating characteristic curves were used for data analysis [22]. E-nose-discriminated patients with fixed asthma from COPD patients with an 88% accuracy (AUC 0.95, 95% CI 0.84–1.00, sensitivity 85%, specificity 90%) and patients with classic asthma from COPD patients with an 83% accuracy (AUC 0.93, 95% CI 0.87–1.00, sensitivity 91%, specificity 90%; both $p < 0.001$) [22]. Patients with fixed asthma and classic asthma could not be discriminated [22].

COPD

A cross-sectional study investigated whether an e-nose could distinguish between 30 patients with COPD, 20 patients with mild to severe asthma, 20 healthy smokers and 20 healthy non-smokers [4]. Breath sampling was performed after a wash-in with VOC-filtered air. The primary analysis was done comparing the breathprints obtained from patients with asthma and patients with COPD. Data were reduced by PCA, canonical linear discriminant analysis was used as a classifier, and cross-validation with the leave-one-out method was used to calculate the cross-validated accuracy value (accuracy) expressed as a percentage. E-nose could discriminate patients with mild-to-severe asthma from patients with COPD (accuracy 96%, $p < 0.001$), healthy smokers (accuracy 93%, $p < 0.001$) and healthy non-smokers (accuracy 95%, $p < 0.001$) [4]. A partial overlapping between patients with COPD and healthy smokers (accuracy 66%, $p = 0.006$) was observed. This might reflect the presence of asymptomatic smokers with normal lung function who are at higher risk for COPD [4]. Longitudinal studies to clarify this are required. Breath-

prints of patients with COPD who were current smokers or ex-smokers were similar (p = 0.16) [4].

In one study including 12 patients with mild (GOLD stage I) COPD and 16 patients with moderate (GOLD stage II) COPD, e-nose breathprints were associated with airway inflammation activity as reflected by sputum eosinophil cationic protein (ECP; r = 0.84, p = 0.002) and myeloperoxidase (MPO; r = 0.72, p = 0.008) in patients with mild COPD [55]. E-nose had high sensitivity and specificity for inflammatory activity in patients with mild COPD as shown by ROC curves (ECP: AUC 1.00; MPO: AUC 0.96) [55]. Validation of the e-nose technology according to the Standards for Reporting of Diagnostic Accuracy guidelines [62] is warranted for establishing its utility for the diagnosis of asthma and COPD.

Lung Cancer

Breath VOC patterns detected by e-nose in patients with lung cancer are different from those in healthy subjects [5, 6, 18–21]. One study with an e-nose included two phases [5]. In a discovery and training phase, breathprints from 14 patients with bronchogenic carcinoma and from 45 individuals who were either healthy subjects or patients with non-cancerous lung disease were non-blindly analysed for investigating possible between-group differences [5]. PCA and canonical discriminant analysis were applied and between-class discrimination was quantified by Mahalanobis distance. GC/MS, performed on breath samples from 8 patients with lung cancer, identified several VOCs, including isopropanol, acetone, pentane and benzene [5], which have been linked to lung cancer [63–67]. Once it was established that patients with lung cancer had different breathprints, the training set was used to build an SVM prediction model that was validated in the second phase of the study and applied prospectively in a group of 14 individuals with lung cancer and 62 individuals without lung cancer, either healthy or with non-cancer lung disease, who were assessed in a blinded manner [5]. The e-nose discriminated between patients with bronchogenic carcinoma and healthy control subjects or control subjects with non-cancer lung disease with a sensitivity of 71% (95% CI 42–92), a specificity of 92% (95% CI 82–97) for detecting lung cancer, a positive predictive value of 67% (95% CI 38–88) and a negative predictive value of 93% (95% CI 84–98) [5]. However, the potential utility of the e-nose for early diagnosis of lung cancer has to be established in population-based screening.

In another cross-sectional study, the discriminant power of e-nose was studied in 10 patients with non-small cell lung cancer, 10 patients with COPD and 10 healthy subjects [18]. Breathprints from patients with lung cancer clustered distinctly from those of patients with COPD (cross validation value: 85%; Mahalanobis distance: 3.73) [18]. The e-nose also discriminated between patients with lung cancer and healthy subjects in duplicate measures (cross validation value: 90 and 80%; Mahalanobis distance: 2.96 and 2.26) [18].

The discriminative ability of LibraNose was tested in 28 patients with lung cancer, 28 patients with non-cancer lung disease and 36 healthy non-smokers [6]. Exhaled breath principally derived from the alveolar compartment was analysed. Partial least squares-discriminant analysis cross-validated by the leave-one-out technique [68] was used for classification. The e-nose distinguished patients with lung cancer from healthy subjects with a sensitivity of 85%, a specificity of 100%, a negative predictive value of 90% and a positive predictive value of 100% [6]. The classification rate in patients with lung cancer and with non-cancer lung disease was 86% with a sensitivity of 93% and 79%, respectively. When breathprints from the 3 groups were analysed, the classification rate was 79% with a sensitivity of 89% for identifying lung cancer, whereas the ability of the e-nose to distinguish patients with non-cancer lung disease from healthy subjects was lower as 9 patients were false negative [6].

In a cross-sectional study including 49 patients with non-small cell lung cancer, 73 patients with non-cancer lung disease and 21 healthy control subjects, a colorimetric sensor array was able to predict the presence of lung cancer with a sensitivity of 73% and a specificity of 72% (p = 0.01) [19].

In a cross-sectional study, alveolar breath obtained from 20 patients with lung cancer (12 smokers, 8 non-smokers), 16 patients with head-and-neck cancer (10 smokers, 6 non-smokers) and 26 healthy subjects (7 smokers, 19 non-smokers) was analysed with NA-NOSE [21]. PCA with ANOVA and t test and SVM with cross-validation were used for data analysis [21]. NA-NOSE discriminates between patients with lung cancer and healthy subjects, and patients with head-and-neck cancer and lung cancer [21]. Specificity and sensitivity, determined through cross-validation, for detecting lung cancer was 100%. Specificity and sensitivity for discriminating between head-and-neck cancer and lung cancer was 100% [21]. Between-group differences observed with chemical sensor arrays were confirmed with GC/MS. A proposed set of breath VOCs that discriminate between lung cancer and head-and-neck cancer include ammonium acetate, 3-methyl-hexane, 2,4-dimethyl-heptane, 4-methyl-octane, p-xylene and 2,6,6-trimethyl-octane

[21]. Smoking seems to have no effect on these VOC patterns [21]. However, studies to formally address this issue are required. It is not known whether breath VOCs identified by GC/MS are the same as those which are responsible for between group discrimination observed with NA-NOSE [21]. Establishing whether this technique can be used as a screening test for lung cancer requires large studies [21].

Tuberculosis

A chemical sensor array consisting of 14 conducting polymer sensors based on polyaniline (Bloodhound 114® E-Nose; Scensive Technologies Ltd., Normanton, UK) was used for detecting mycobacterium tuberculosis in human sputum [69] and for distinguishing between mycobacterium tuberculosis and other pathogens in culture and in spiked sputum samples [70]. A similar e-nose consisting of 16 sensors detects and identifies 12 different microorganisms by analysing the volatile compounds produced by the plate cultures [71].

Sinusitis

E-nose sampling of nasal exhalation from patients with suspected sinusitis is able to predict correctly the diagnosis of sinusitis in at least 72% of the samples [72]. A colorimetric sensor array can be used to distinguish breath samples from patients with chronic bacterial sinusitis versus healthy control subjects with 90% accuracy [7]. A chemical sensor array identifies biofilm- versus non-biofilm-producing *Pseudomonas* and *Staphylococcus* species with accuracy ranging from 72.2% to 100% [73]. Specific VOCs have been identified in infected sinus mucus samples with GC/MS [74]. Other potential applications of e-noses include identification of patients with chronic rhinosinusitis through analysis of nasal out-breath samples (classification rate of 80%) [75].

Cystic Fibrosis

Using e-nose technology based on MS, breath volatile compounds have been measured in patients with cystic fibrosis and compared with healthy subjects [56–58].

Other Diseases or Pathophysiological Conditions

Methodologies based on e-noses are able to detect bacterial pathogens of the upper respiratory tract from in vitro samples [76] and swab samples from patients with ear, nose and throat infections with a classification rate of 88.2% [77]. E-nose analysis has been applied to the identification of ventilator-associated pneumonia in patients in surgical intensive care units [8, 78, 79].

Limitations

Standardization and development of a robust methodology is the priority in e-nose research. Likewise, improvement in sensor specificity, sensitivity and long-term stability is essential for the development of this technology. At present, chemical sensor arrays, which are commonly used as e-noses, are not sufficiently sensitive to detect those breath VOCs in the low ppb range, can be affected by ambient conditions (e.g., humidity and temperature), and have a limited life time. Being selective for patterns of volatiles (breathprints), sensor arrays are not suitable for identifying and quantifying the single VOCs in exhaled breath, which requires different e-nose techniques such as GC/MS. Studies aiming at identifying the complete profile of biomolecules ('breathome') in the gaseous and liquid phase of the exhaled breath with MS techniques and nuclear magnetic resonance spectroscopy [80–82] are on-going.

The fact that some well-performing chemical sensor arrays (e.g., LibraNose, NA-NOSE) are prototypes currently limits the development of this technique. Lack of studies comparing different devices in the same subjects and differences in data analysis make it difficult to compare results from different centres. In one study with an e-nose, data were externally validated [22]. Similar studies in larger cohorts of patients with respiratory disease are required. Procedures for building a model based on data obtained with one e-nose and applied to data collected with a different e-nose have been proposed (e-nose data mapping) [83]. Under controlled experimental conditions and limited to three selected volatiles (ethanol, hexane and ethyl acetate), e-nose data mapping showed similar results, indicating that one nose can be used for the training set and the other for the testing of the model [40]. However, future studies to validate this strategy in clinical trials when complex breath volatile mixtures are analysed should be undertaken. Comparison of different e-noses is part of a European Union Innovative Medicines Initiative project on severe asthma, called Unbiased Biomarkers for the Prediction of Respiratory Disease Outcomes (U-BIOPRED). A platform of several e-noses will be used for analysing breath samples from asthma patients and healthy subjects at the same time. Data will be analysed with the same algorithms and integrated with data from other 'omics' platforms using a systems biology approach (http://www.fp7-consulting.be/en/ubiopred/).

The ideal all-purpose e-nose does not exist. The choice of the 'right' breath sampling protocol and e-nose will much depend on the required applications which should

take into consideration the physical-chemical properties of the breath VOC to be studied (e.g., molecular weight, hydrophilia, polarity), the type of respiratory disease (e.g., respiratory tract, small airway, interstitial, alveolar disease) and the characteristics of patients with respiratory disease (e.g., pulmonary function testing, breathing patterns).

The available studies on the applications of the e-nose in respiratory medicine are limited to relatively small groups of patients with a known diagnosis. The role of the e-nose in population-based screening for respiratory diseases has to be established in large prospective studies.

Complex data analysis requiring trained statisticians and cost are other current limitations for large-scale use of the e-nose technique. Chemical sensor array data generally require multivariate statistical analysis for pattern recognition, whereas MS techniques are not suitable for routine use. Basically, the e-nose technique has to be validated and made simple before it can be considered for clinical applications.

Conclusions

The e-nose discriminates between patients with respiratory disease and healthy control subjects and also among patients with different respiratory diseases, and is emerging as a new, non-invasive diagnostic tool. E-noses are handheld, portable devices that may provide immediate results, but breath VOC pattern recognition often requires off-line analysis with multivariate statistical algorithms. Combined technologies, which exploit the high sensitivity of chemical sensor arrays with the high specificity of GC/MS, can be used for identifying selective breath volatile compounds. This non-invasive approach to the assessment of pulmonary inflammation might lead to a better understanding of the pathophysiology of lung disease and have implications for the diagnosis, pharmacological treatment and management of patients with respiratory disease. However, e-nose technology has limitations that need to be overcome before its potential as a clinical tool can be considered.

Future work will involve establishing the detection limit for breath VOCs, marketing and comparing different e-noses, identifying and quantifying breath VOCs responsible for between-group differences, improving breath sample stability and performing between-laboratory validation studies of this technique. On the clinical side, longitudinal studies to establish the utility of e-nose for monitoring disease progression and controlled studies to ascertain its potential role for assessing pharmacological response are warranted.

In combination with other 'omics' platforms, e-nose technology might contribute to the identification of subphenotypes of patients with respiratory diseases and new surrogate markers for respiratory disease, provide a molecular basis to a personalized pharmacological treatment, and facilitate the development of new drugs. As part of a non-invasive integrated approach, e-noses might contribute to a better management of individual patients with respiratory disease.

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