EDITOR'S PICK

An electronic nose is a small, portable device that can detect, with reasonable accuracy, the breath 'fingerprints' of various respiratory diseases. It detects volatile organic compounds in exhaled air and can differentiate between common respiratory illnesses. However, larger studies are needed in order to evaluate and standardise the device.

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THE ELECTRONIC NOSE ARISES INTO THE 21st CENTURY

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ABSTRACT

Electronic noses (eNoses) are instruments designed to imitate the sense of smell. These devices are used to detect and precisely distinguish odours within complex samples at a relatively low cost, and these properties make them very useful in a diverse range of clinical scenarios. An eNose is typically composed of a multisensor array, an information-processing unit, and a pattern-recognition algorithm. The multisensor array combines to respond globally to a wide range of volatile organic compounds (VOCs) and the output provides a distinct digital response pattern. Clinical 'breathprints' or 'smellprints' contain VOCs and respiratory diseases such as chronic obstructive pulmonary disease (COPD), asthma, and lung cancer can be detected by this novel technique. Moreover, patients with exacerbated COPD and a positive microbiological culture can be differentiated from those with stable disease. The eNose displays high accuracy in detecting obstructive sleep apnoea syndrome, and common conditions in the intensive care unit such as acute respiratory distress syndrome and ventilator-associated pneumonia have also been studied in relation to the use of eNoses. Information contained within breathprints interpreted by eNoses may serve as non-invasive biomarkers in respiratory medicine and infectious diseases, as well as other branches of medicine.

Keywords: Electronic nose (eNose), volatile organic compounds, asthma, chronic obstructive pulmonary disease (COPD), lung cancer, obstructive sleep apnoea syndrome, ventilator-associated pneumonia, a cute respiratory distress syndrome.

INTRODUCTION

The widespread adoption of technology across almost all fields of medicine represents a key point in the evolution of many diagnostic procedures and therapeutic options. The electronic nose (eNose) is an example of cutting-edge technology adapted to provide a new medical tool. Exhaled breath from individual patients contains volatile organic compounds (VOCs) that can provide

important information. For example, 'smellprints' can be used to identify different conditions or processes occurring inside an organism. Smellprints are the result of analysis by a series of nanosensors contained within the eNose, these sensors are capable of detecting VOCs. The electrical resistance of these sensors changes specifically when exposed to volatile particles, which generates a signal that can be interpreted using various methods. Using mathematical algorithms, the eNose is trained to recognise smellprints through a process of comparison with previously recorded patterns. Data analysis includes a range of software, including pattern-recognition programs in MATLAB (v.R2012a) and SPSS, amongst others. Data obtained from pattern recognition can be analysed throughout cross validation, principal component analysis, and canonical discriminant analysis.

The basic setup for eNose sampling usually consists of a specific device containing the nanosensors, software for data interpretation, a collecting bag containing VOC-filtered ambient air that serves as a baseline, and a collecting bag containing the breath sample. The collecting bags are connected to the eNose through a purge inlet (baseline air) and a sample inlet (sample air) using a syringe as a connector between the bag and the eNose device. The eNose software allows several settings for sample processing. eNoses are handheld, portable devices that provide immediate results and, therefore, breathprints are rapid, non-invasive, cost-effective, and easy to perform compared with other methods currently available for analysing exhaled breath. These characteristics are important for the future clinical applicability of eNoses.

THE eNOSE IN OBSTRUCTIVE AIRWAY DISEASES

The most common obstructive lung diseases, chronic obstructive pulmonary disease (COPD) and asthma, share some common characteristics but differ in terms of treatment options and expected morbidity and mortality outcomes. Fens and colleagues[1] investigated whether breathprints from 30 patients with COPD, 20 asthmatic patients, 20 healthy smokers, and 20 healthy non-smokers could be differentiated using an eNose (Cyranose 320®; Smith Detections, Pasadena, CA, USA). Their results showed an accuracy of 96% ($p<0.001$) when discriminating between asthma and COPD samples, an accuracy of 95% ($p<0.001$) when discriminating between asthma and non-smoking control samples, and 92.5% ($p<0.001$) when discriminating between asthma and smoking control samples.

Asthma is characterised by airway inflammation; this pathophysiological base makes it an obvious condition in which to investigate potential non-invasive biomarkers, such as VOCs. A study including 10 young patients with mild asthma, 10 young controls, 10 older patients with severe asthma, and 10 older controls investigated whether an eNose (Cyranose 320) was able to differentiate between these groups.[2] The smellprints of patients with mild asthma could be fully differentiated from young controls (cross-validation value [CVV]: 100%), and patients with severe asthma could be distinguished from older controls (CVV: 90%). However, the accuracy of the eNose was lower when differentiating between patients with mild and severe asthma.

One of the many challenges in COPD is the assessment of eosinophilic inflammation used to identify subgroups of patients in whom the use of inhaled corticosteroids may be most effective. A sputum cell count is not always available; it is a time-consuming procedure and can be uncomfortable for the patient. In order to improve this situation, differential cell counts and airway inflammatory markers were obtained through induced sputum samples from 12 mild and 16 moderate COPD patients classified according to the former Global Initiative for Chronic Obstructive Lung Disease (GOLD) criteria.[3] The eNose breathprints were highly associated with airway inflammation in the group of mild COPD patients, this association was not found in the moderate COPD group. Thus, breath analysis may be used for assessment and monitoring of airway inflammation in COPD.

A preliminary study by our group investigated the hypothesis that an eNose could identify infection-associated VOC patterns in breath samples from patients experiencing acute exacerbation of COPD (AECOPD), and differentiate these patterns from those obtained from COPD patients without exacerbation.[4] 53 patients with AECOPD, 18 with COPD with associated pneumonia, and 29 with stable COPD were enrolled in the study. Sputum samples for microbiological evaluation were obtained from all patients and exhaled air samples were processed by an eNose (Cyranose 320).

Breathprints from AECOPD patients were distinguishable from stable COPD in the presence of microorganisms (p=0.036; 84.4% sensitivity; 70.0% specificity), as well as in the absence of infection (p=0.014; 90.6% sensitivity; 80.0% specificity). However, breathprints from COPD patients with pneumonia failed to show a significant difference from AECOPD samples in the presence of microorganisms (p=0.097), but were distinguishable in the absence of infection (p=0.013; 87.5% sensitivity; 77.8% specificity) and from stable COPD (p<0.05; specificity 90.0%). Different breathprints related to positive infection can be detected by the eNose. In addition, breathprints obtained from AECOPD patients with or without pneumonia could be distinguished from those obtained from stable COPD patients, even in the absence of a positive microbiological culture.

In a proportion of patients with COPD, bacterial colonisation of the airways increases the rate of mortality due to augmented airway inflammation, and increases the frequency and severity of exacerbations. Sibila et al.[5] performed a study to evaluate the ability of an eNose (Cyranose 320) to discriminate between COPD patients with and without airway bacterial colonisation, determined by quantitative culture of specimen brush, the gold standard for the diagnosis of distal airway infection. 13 healthy controls and 37 clinically stable moderate-to-severe COPD patients (colonised versus non-colonised) were studied. Canonical discriminant analysis showed an accuracy of 89% (p<0.001) with a sensitivity of 89% and a specificity of 96% when discriminating between colonised and non-colonised COPD patients.

THE eNOSE IN CANCER

The eNose has been evaluated for the detection of smellprints from patients with COPD and lung cancer (LC). A cross-sectional study[6] included 10 patients with non-small-cell LC, 10 patients with COPD, and 10 healthy controls. Smellprints from LC patients were distinct from those of patients with COPD (CVV: 85%). In duplicate measurements, the eNose distinguished patients with LC from healthy controls (CVV: 90% and 80%, respectively). Machado et al.[7] performed a two-phase study in which the ability of an eNose to discriminate between samples from 14 patients with bronchogenic carcinoma and 45 healthy controls was demonstrated during the first phase. During the second phase of the study, a cancer prediction model was created and applied to a separate group of 14 patients with LC and 62 without. The results from the validation study showed that the eNose displayed a sensitivity of 71.4% and a specificity of 91.9% for the detection of LC.

Asbestos inhalation is associated with malignant mesothelioma and other respiratory diseases. A non-invasive screening tool for high-risk populations would be useful for detecting mesothelioma during the early stages of the disease. Using an eNose (Cyranose 320) and applying principal component analysis, two contemporary studies by Dragonieri et al.[8] and Chapman et al.[9] have reported almost identical results, with high levels of discrimination between samples from patients with malignant mesothelioma, patients with long-term asbestos exposure, and patients with other asbestos-related non-malignant diseases.

Another example of a rapid, diagnostic, clinically applicable, and non-invasive use of an eNose is in the detection of prostate cancer.[10] Urine headspace samples from pre-operative patients with prostate cancer have been compared with those from benign prostatic hyperplasia patients using an eNose (ChemPro® 100); the results using leave-one-out cross-validation reached a sensitivity of 78%, a specificity of 67%, and an area under the curve of 0.77. Head and neck squamous cell carcinoma (HNSCC) has also been evaluated using an eNose, with a sensitivity of 90% observed when discriminating between VOC patterns from 36 HNSCC patients and those from 23 controls with benign conditions.[11] Colorectal cancer (CRC) treatment and prognosis depends on the diagnostic stage of the disease. The VOC patterns obtained from the gas in stool samples collected from patients undergoing a colonoscopy have also been analysed using an eNose. A sensitivity of 85% and a specificity of 87% were observed when discriminating between smellprints obtained from 40 patients with confirmed CRC and those obtained from 60 patients with advanced adenomas as well as 57 healthy controls.[12]

SMELLING OTHER RESPIRATORY DISEASES

Differentiation of samples from healthy controls from those obtained from patients with obstructive

sleep apnoea syndrome (OSAS) has also been investigated using an eNose. Greulich et al.[13] analysed VOC patterns in this very common disease that is associated with an increased risk of cardiovascular events and metabolic disorders. The diagnosis of OSAS is currently dependent on expensive, not always available, and time-consuming techniques such as polysomnography or respiratory polygraphy. The study included 40 OSAS patients and 20 healthy controls, with asthma and COPD patients excluded. The results of the study showed a high sensitivity (93%) and specificity (70%), with the corresponding area under the receiver operating characteristics curve of 0.85 (95% confidence interval: 0.75–0.96). Moreover, changes in breathprints after 3 months of uncontrolled continuous positive airway pressure therapy confirmed a relationship between the OSAS disease process and the VOC patterns detected by the eNose. The high accuracy of this tool might suggest it represents a non-invasive, portable, and cheap diagnostic method suitable for the diagnosis of OSAS.

Breathonomics has also been used to assess the identification of pulmonary embolism (PE) in a proof-of-principle study[14] including 20 patients with confirmed PE and 20 patients in whom this diagnosis was excluded. Patients were categorised according to the presence or absence of comorbidities and exhaled breath samples were analysed using an eNose (Cyranose 320). For the non-comorbid group, PE and non-PE samples were differentiated with an accuracy of 85% (17/20 correctly classified, p=0.008), the positive predictive value was 0.86, and the negative predictive value was 0.83. However, the accuracy in the comorbid group was 65% (p=0.78), which demonstrates the confounding effect of comorbidities on the interpretation of breathprint data.

A strict follow-up on lung transplant recipients is necessary in order to prevent serious complications and invasive techniques are part of this process. Lung transplantation has also been a setting in which the eNose has been investigated. Invasive techniques are, at the moment at least, the only methods available to detect and monitor post-transplantation complications, such as organ rejection and infections, throughout the post-operative period; unfortunately, these invasive techniques are frequently associated with complications. Kovacs et al.[15] followed 16 patients receiving a lung transplant and compared them with healthy controls. When principal component analysis was applied, an eNose (Cyranose 320) was able to discriminate between the two patient groups with an accuracy of 73% (p<0.001), a sensitivity of 63%, and a specificity of 75%. These differences in the exhaled volatile compounds might be explained by the systemic or local airway changes ongoing in lung transplant receivers. An association between plasma levels of the immunosuppressant drug tacrolimus and the VOC patterns obtained from lung transplant recipients was also reported.

THE eNOSE AND INFECTIONS

Ventilator-associated pneumonia (VAP) is the most common infection in the intensive care unit that is associated with an increased risk of patient death. In a prospective comparative study, the ability of an eNose to identify VAP through analysis of bronchoalveolar lavage fluid was investigated in 44 VAP patients and 6 controls.[16] The eNose was able to correctly identify 77% of the VAP samples, with the accuracy being comparable with accepted microbiological techniques.

In an attempt to diagnose and classify acute respiratory distress syndrome (ARDS) based on pulmonary injury, inflammation, and bilateral rich pulmonary oedema, VOC patterns from mechanically ventilated intensive care patients were analysed within 24 hours of admission. Cases of ARDS were classified according to the Berlin definition as ‘mild’, ‘moderate’, or ‘severe’. A commercially available eNose (Cyranose 320) was trained using sparse-partial least square logistic regression with a 10,000-fold cross-validation to select variables and limit false positives. A sensitivity of 91% and a specificity of 62% were obtained when discriminating between moderate and severe ARDS, and the control group confirmed the results by temporal external validation. Modestly accurate diagnostic results were found when attempting to discriminate between patients with ARDS and patients with pneumonia and cardiac pulmonary oedema (CPO), although the breathprints of patients with pneumonia and CPO could be differentiated from those obtained from patients with moderate or severe ARDS with a greater degree of accuracy.[17]

A small number of patients experiencing prolonged chemotherapy-induced neutropaenia were assessed through the analysis of exhaled breath to determine invasive aspergillosis.

The sensitivity and specificity were 100% and 83.3%, respectively. The high mortality rate of pulmonary aspergillosis can be reduced by timely diagnosis. eNose technology (Cyranose 320) could enable the detection of invasive aspergillosis at an earlier stage compared with available diagnostic tools by principal component analysis. VOC patterns from patients with prolonged chemotherapy-induced neutropaenia are different and can be detected via eNose technology.[18]

Active tuberculosis remains a major global health problem and many diagnostic techniques are not available in rural areas. A study has been carried out to determine the diagnostic accuracy of an eNose (DiagNose, C-it BV) when used to identify tuberculosis infection using exhaled breath. The proof-of-principle study[19] involved 30 patients and reported a sensitivity of 95.9% and specificity of 98.5%, whereas a validation study including 194 participants reported a sensitivity of 93.5% and a specificity of 85.3% when discriminating tuberculosis patients from healthy controls.

Breathprints from 64 paediatric patients suffering from cystic fibrosis (CF) were analysed by an eNose (Cyranose 320) in order to investigate potential differences when compared with samples from 21 patients with primary ciliary dyskinesia and 21 healthy volunteers, with statistically significant results being reported. Moreover, VOC patterns from CF patients with chronic *Pseudomonas aeruginosa* infection differed significantly from those obtained from non-chronically infected CF patients.[20]

Faecal VOC patterns from paediatric patients were studied by an eNose in order to investigate the potential detection of inflammatory bowel disease (IBD). Samples from IBD patients during both active disease and remission were compared with samples obtained from a control group.[21] Patients with IBD were further categorised according to whether the patients received a diagnosis of ulcerative colitis (UC) or Crohn´s disease (CrD). Smellprints from patients with UC and CrD could be discriminated from each other as well as from controls. In addition, UC samples could be differentiated from CrD samples during both active disease (sensitivity of 97%, specificity of 92%) and during clinical remission.

Smellprints from tracheal aspirates (TAs) were obtained from mechanically ventilated pre-term neonates and analysed by an eNose (Cyranose 320) in order to distinguish acutely infected from non-infected patients, as well as identify the presence of bronchopulmonary dysplasia (BPD).[22] VOC patterns from patients with laboratory-confirmed bloodstream infections were different from those without infection regardless of a positive ($p<0.0001$) or negative microbiological culture from the TA sample ($p<0.0001$). Smellprints from patients who had BPD were different from those without BPD. The authors conclude that the simple and rapid eNose technique could be a useful tool to determine VOC patterns in TA samples as a diagnostic marker in pre-term neonates.

LIMITATIONS AND CONCLUSION

Great expectations surround the potential utility of the eNose for the diagnosis of various medical diseases. Being a simple, non-invasive, and transportable technique that offers quick access to results, the eNose represents a compelling piece of technology from a medical research point of view. Published research would suggest that, during the early stages of various diseases, VOC patterns may play a key role in the phenotyping of patients. In addition, the eNose may play a role in offering a more effective, personalised approach to therapy in the future.

One of the main limitations of the eNose technique is the absence of a standardisation related to the different methods used in the collection of VOC samples and the generation of smellprints. Indeed, expiratory flow rate, breath-holding capacity, and anatomic dead space were studied to determine if the method of collection affected results obtained from exhaled breath samples from LC patients and controls. Differences were found between the results obtained using the two methodologies, mainly in the control group where VOC patterns might be influenced by the technique applied for sample collection.[23]

The interpretation of VOC patterns requires various statistical analysis and software applications, which is an important barrier that must be overcome in order for the technology to be embraced in future daily clinical practice. Small groups of patients with a known diagnosis have been included in the published eNose studies, but large-scale studies are needed in order to validate the different commercial eNose devices available, the stability of the biological samples obtained, and the reproducibility of intra and inter-laboratory sample measurements. Prospective

studies including population-based screening for respiratory diseases should be a key point in determining the potential applicability of the eNose to daily clinical activity.

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