Breath Analysis of Lung Cancer Patients Using an Electronic Nose Detection System

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Abstract— ***Background.*** The measurement of gaseous compounds in exhaled breath, such as volatile organic compounds (VOCs), may provide a non-invasive technique for assessing lung pathology, and some of which are associated with lung cancer (LC). VOC analysis is laborious while electronic noses are emerging as rapid detectors of an array of gaseous markers recognising a characteristic 'smellprint'.

Objectives. To conduct a pilot breath analysis using an electronic nose to test the hypothesis that there would be significant differences in the smellprint patterns between newly diagnosed LC patients and control subjects.

Methods. Eighty-nine subjects were recruited, consisting of non-smokers (33), ex-smokers (11), smokers (18), patients with respiratory disorders (11) and LC patients (16). Subjects exhaled into gas-impermeable bags for off-line eNose measurements with a six-channel electronic detection module ENS Mk 3 (E-Nose Pty, Sydney). The time:response curve from each channel was evaluated for four parameters: rate to peak height, peak height, rate to recovery and area under the curve.

Results. The results showed significant differences between lung cancer and control groups when measuring peak height in channel 1 ($p=0.025$); rate to recovery in channel 3 ($p=0.045$); and rate to peak height in channel 3 ($p=0.001$).

Conclusion. The results show promise in that there were significant differences in the smell-print of subjects with lung cancer compared with control subjects. Further standardisation of the technique will assist in improving the sensitivity and specificity of the method, with potential to use the analysis in a number of diseases where characteristic signatures occur in the breath.

***Index Terms*— Exhaled breath analysis, electronic nose, lung cancer**

I. Introduction

Lung cancer is usually diagnosed at an advanced stage and as a result patients consequently have poor survival rates. Early stage disease is amenable to curative surgery, but to date no screening process has been able to detect disease at a stage which has altered the overall survival. Current methods of detecting lung cancer such as computed tomography scans are time-consuming, expensive and require invasive confirmation of the diagnosis. There is a need for tests which are capable of early lung cancer detection, particularly as the at-risk population is clearly defined as those who are current or ex-smokers.

Exhaled breath analysis is becoming an increasing area of interest for studying the respiratory system and function. The exhaled breath contains over 250 chemical entities including nitric oxide, carbon monoxide and volatile organic compounds (VOCs) [1]. Exhaled breath has been analysed in non-malignant respiratory disorders such as chronic obstructive pulmonary disease (COPD), asthma, bronchiectasis, cystic fibrosis and pulmonary fibrosis [2-5]. Measurement of VOCs in the gaseous phase of exhaled breath has become an area of increasing research including in lung cancer [6-8]. Some are now in early clinical development [8]. Other adaptations of the VOC methods include solid phase micro extraction (SPME) which is a virtual array of surface acoustic wave (SAW) gas sensors with an imaging recognition technique. possible biomarkers indicative of pulmonary carcinogenesis [9].

VOC analysis is laborious requiring extraction processes and control analysis of ambient air, while electronic noses are emerging as having the ability to detect an array of gaseous markers in real time recognising a characteristic 'smellprint'. The electronic nose is a device which detects a chemical reaction which is then converted into an electrical impulse. The term covers many different instruments with fundamentally different principles of operation, and within a group there are very different characteristics.

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The electronic nose is an electronic detection system consisting of an array of coated sensors, e.g. silicon chips that can detect VOCs. The analyser is able to produce smell "fingerprints" of various compounds depending on variables such as chemical reactivity or electrical properties. Breath samples are channelled through the machine, aided by a negative pressure gradient to reach the sensors. Depending on the device, multiple sensors acquire data, with additional sensors for ambient temperature and humidity.
This can be by coating electrodes with reactive compounds that then change their characteristics or generate heat, and thus alter the electrical conductance, generating a change in resistance which can be displayed as a signal. Most sensors currently use variations on a particular group reaction to increase the number of sensors, but by adding a variety of chemical interactions may further enhance the identification of different 'smellprints'.
Multiple electrodes with different coatings can increase the sensitivity of the device, but in general terms there is a limit to the number of electrodes which will increase sensitivity. One such device has been the Cyranose (Smith's Systems, Watford, UK) which has been shown to be able to identify characteristic signals in the breath of those with lung cancer, although the study was flawed by an unusual control group (i.e. patients with berylliosis, and no control group of otherwise normal smokers) [10]. Other devices have used systems which incorporate other technologies [11].

The eNose (ENS Mk 3, E-Nose Pty, Sydney, Australia) has a track record of successful applications to industries such as meat processing, food, wine and sewage [12]. Abattoirs and sewage farms, for example, are sites where collection of preliminary data can create a database of acceptable and unacceptable odour levels. When elevated levels are reached, the pattern is automatically recognised and can activate a set procedure to rectify the problem. Its use in industry has stimulated interest in its possible medical application, such as the monitoring of respiratory conditions [11, 13, 14].

Recent studies have used this system to analyse gaseous and other compounds found in exhaled breath [15]. Preliminary electronic nose analysis has shown significant differences between control groups and lung cancer patients, with one study describing a sensitivity of 71.4% and specificity of 91.9% for lung cancer [16]. Di Natale et al. employed a device comprised of quartz microbalance gas sensors coated with different metalloporphyrins [16]. These sensors were able to detect unspecified alkanes and aromatic compounds, believed to be indicative of lung cancer [16]. A different electronic nose (Cyranose, Smiths Detection, Watford, UK) was used to compare lung cancer patients were compared with 62 other subjects. There was a positive predictive value of 66% and a negative predictive value of 92% [10].These results have indicated the potential of the electronic nose as a non-invasive tool for the early diagnosis of lung cancer, as well as a tool for monitoring the effectiveness of treatment. It was hypothesised that the electronic nose will be able to define distinct signature patterns from each subject group which will distinguish between lung cancer versus control groups

II. MATERIALS AND METHODS

A. Subjects and study design

The design was a cross-sectional, observational study of a cohort of subjects with newly diagnosed lung cancer prior to any treatment, and healthy non-smokers, ex-smokers, smokers, and patients with respiratory conditions, matching for age, gender and smoking history where appropriate. Lung cancer patients were recruited from the Multidisciplinary Lung Cancer Clinic, and the Respiratory Medicine and Oncology departments at the Prince of Wales Hospital. Control subjects comprised respiratory clinic patients, research and hospital staff, patients' relatives and local residents.

Subjects were recruited to the following groups with the following criteria:
i. Non-smokers: Have never smoked, or have smoked less than 1 pack year and have no known diseases nor are taking any medication;
ii. Ex-smokers: Have ceased smoking for at least one year or more;
iii. Smokers: Current smokers;
iv. Respiratory disorders: Patients who have conditions such as asthma, pleural effusion, COPD and bronchiectasis. These were included to provide subjects who could develop lung cancer but who currently have other inflammatory lung diseases;
v. Lung Cancer: Patients who have been diagnosed with primary cancer of the lung and confirmed by cytology or histopathology, but who had not yet undergone any treatment and/or therapy.
After informed consent was obtained, a questionnaire was administered regarding medical conditions, current medications and smoking history, such as pack years.

B. Breath analysis

Oral exhaled breath was collected in a 2-litre inert gas impermeable bag without nose clips as the use of noseclips can contaminate breath with nasal gas by opening the nasopharyngeal velum [17]. Two bags were filled to assess reproducibility. The gas from each bag was analysed by the eNose in duplicate, resulting in a total of four graphic displays per subject. Breath samples were stored at 4ºC but were analysed within 4 hours.

The E-Nose© (E-Nose Mk2© and Mk3©, E-Nose Pty. Ltd, Australian Technology Park, Sydney) is an electronic detection system with an array of 6-channel coated chip sensors, each consisting of tin oxide electrodes with individualised highly-reactive rare earth coatings. These lanthanide element coatings have been shown to undergo oxidisation which is detected by a chip-based microelectronic device as a change in resistance. The change in voltage is recorded simultaneously for all channels. An additional two channels provided measurements of temperature and humidity.

An electronic memory is generated allowing the identification of similar patterns between samples rather than the identification of specific compounds. The data logger acquisition software (PicoLog for Windows, Pico Technology Ltd.) samples and digitises the voltage output of each channel simultaneously to generate graphic peaks. For each display, four variables were determined and compared between groups: 1) the peak height of the curve; 2) rate to peak height; 3) rate of recovery; and 4) area under the curve.
After each sample had been analysed, the signal for each channel was required to return to baseline before the next sample was analysed.

C. Ethics

The study protocol was approved by the Research Ethics Committee of South Eastern Sydney Area Health Service and St Vincent's Hospital Human Research Ethics Committee.

D. Statistical analysis

eNose data conformed to the Normal distribution and were analysed using ANOVA and unpaired t-tests. Principal Components Analysis (PCA) was performed to extract a subset of parameters for further analysis. Statistical analyses were performed using SPSS 12.0.

III. Results

Newly diagnosed lung cancer patients (16), and control subjects (healthy non-smokers (33), ex-smokers (11), smokers (18), and patients with respiratory conditions (11)) were recruited in this study. Subject characteristics are summarised in Table 1.
Controls were amalgamated into a single control group as there was no significant differences between the subgroups for various parameters tested.
PCA was performed and Channels 1-4 for "Rate to peak height", Channels 1-3 for "Peak height" and Channels 1-3 for "Rate of recovery" were extracted for further analyses.

A. Rate to peak height

Patients with lung cancer had significantly lower rates to peak height for Channel 1 when compared with control subjects (6.76 ± 1.66 mV/s vs 19.05 ± 3.22 mV/S, p=0.001, Figure 1).

B. Peak height

Lung cancer patients showed different patterns of response for Channel 1 comparing the peak height of the curves. Lung cancer patients had significantly lower peak height values for Channel 1 when compared with control subjects (434±79 mV vs 640±43 mV, p=0.025, Figure 2).

C. Rate of recovery

Significant differences were seen when comparing the rate of recovery for Channel 3, with lung cancer patients having significantly lower rate of recovery values when compared with control subjects (8.04±1.26 mV/s vs 10.62±0.58 mV/s, p = 0.045, Figure 3).

D. Area under curve

No significant differences were seen for lung cancer patients and control subjects when comparing area under the curve values.

IV. Discussion

Exhaled breath analysis in the study of lung cancer has been an increasingly exciting field in recent years with mounting evidence that patterns of gaseous and non-gaseous markers can be identified in the exhaled breath of lung cancer patients [18-21]. There is, therefore, a continual search for biomarkers of this disease and eNose could potentially be an effective tool given its ability in detecting smellprints of individuals and the non-invasive nature of the sampling process.

The results from this study add further weight to the argument that it is possible to distinguish breath patterns between the study groups, separating those with lung cancer from other appropriate control groups. To date, the use of appropriate controls has been lacking, with either inappropriate control subjects (e.g. patients with berylliosis) or a lack of smokers and ex-smokers, together with not studying those with the most common smoking-related lung disease, COPD [10]. From these preliminary results it should be possible to train the eNose for breath analysis to detect cancer versus non-cancer. This would allow further evaluation with the derivation of receiver - operator curve analysis in a larger study group. In addition, these data will allow sample size calculations to be performed to enable further study to be sufficiently powered.

One factor which emerged was that the control group was well matched for age within the subgroups, but not for age in the LC group. It is, however, not known if age is an independent variable in breath analysis using this device.

One of the strengths of a device such as the eNose is that it can be "trained" to recognise a very large number of factors which together complete the identifiers for a particular disease or condition of interest. This allows it to perform at speed unlike the usual approaches to laboratory analysis which measure a specific single compound with appropriate standard curves and internal quality controls. This overview provided by electronic noses also means that the approach has its limitations: there can be no immediate link with pathogenesis as the mediators or products of the disease are intermingled in the pattern of the signal output, thereby necessitating the definition of a disease for each eNose analysis.

A number of technical problems were encountered during the study. Drift occurred in the signal output which could be related to a number of issues such as volatile components of the ambient air or the performance of the detectors. Activated charcoal filters might be useful for providing VOC and nitric oxide free air for the subjects to inhale and may reduce reducing drift, helping to stabilise the baseline. A more stable baseline would perhaps improve the reproducibility and may

be able to set the eNose machine to an arbitrary 'zero'. Likewise, using VOC-free air or VOC inspiratory filters may also be an option to improve sensor stability.

There are variations in the methods used for measuring breath VOC [22], and a study of volatile organic compounds present in passive smokers as well as active smokers has yet to be determined, and in the future additional measures may be established to reduce variability within individual sampling [23]. Some studies use extraction systems to sample both the breath and also the ambient air, with the air signal being subtracted from the breath sample [8]. In support of the findings described in this study, specific patterns of VOCs have now been described using GC-MS [24].

This preliminary study has demonstrated several differences in the patterns of eNose signals between lung cancer and control group, thereby further stating its potential as a screening tool for detecting smellprints of lung cancer. However, further study which will be sufficiently powered should be conducted to detect further significant differences and uncover a unique smellprint for the early detection of lung cancer. It may also be useful to consider combining eNose analysis of exhaled breath with other methods of exhaled breath analysis (exhaled nitric oxide, exhaled breath condensate) to further improve the accuracy of the battery of tests.

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Figures and tables

Figure 1. Patients with lung cancer had significantly lower rates to peak height for Channel 1 when compared with control subjects (6.76 ± 1.66 mV/s vs 19.05 ± 3.22 mV/S, p=0.001).

Figure 2. Patients with lung cancer had significantly lower peak height values when compared to control subjects (434±79 mV vs 640±43 mV, p=0.025).

Figure 3. Patients with lung cancer had significantly lower rate of recovery values when compared with control subjects (8.04±1.26 mV/s vs 10.62±0.58mV/s, p = 0.045).

	Control Subjects				**Lung Cancer**	**Total**
	Non-smoker	**Ex-smoker**	**Smoker**	**Respiratory Disorders**		
n	33	11	18	11	16	89
M/F	20/13	5/6	12/6	4/7	11/5	

Table 1. Subject characteristics in each of the study subgroups.