

Electronic Nose Prediction of a Clinical Pneumonia Score: Biosensors and Microbes

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Background: The authors performed a prospective study to determine whether breath test analysis using an electronic nose correlates with a clinical pneumonia score.

Methods: Exhaled gas was sampled from the expiratory limb of the ventilator in mechanically ventilated surgical intensive care patients and assayed with the electronic nose. Components of a clinical pneumonia score were recorded concurrently.

Results: The score predicted by the electronic nose showed good correlation with the actual pneumonia score ($r^2 = 0.81$). Bland Altman analysis showed a mean bias of 0.0 (limits ± 2.6).

Conclusions: The electronic nose is a new biosensor technology that correlates with a clinical pneumonia score.

THE sense of smell was one of the first diagnostic tools used by physicians, and terms such as *fetor hepaticus* and *fetor oris* attest to its ancient origins. As an example, the smell of acetone on the breath suggests ketosis, ammonia suggests uremia, and a bitter almond aroma suggests cyanide poisoning. Similarly, skilled microbiologists can often identify plated bacterial organisms by their smell.

In the past several years, devices characterized as electronic noses (e-noses) have become commercially available. Although the underlying technology varies, the general principles are similar to those of the human nose.^{1,2} The archetypal e-nose (fig. 1) has an array of nonspecific chemical sensors that bind to volatile chemicals in the vapor headspace over a sample,³ where the headspace is defined as a measurable volume of gas. The binding is typically reversible. The nature and relative ratio of the molecules in the headspace determines the response pattern of the e-nose sensor array. As a result, the headspace chemistry of a given sample can be represented as a point in multidimensional space (where the number of sensors determines the number of dimensions). Using pattern matching computer algorithms such as neural networks, the electronic nose can be trained to “recognize” unknowns after exposure to known samples.

Based on previous work showing that an e-nose can distinguish among plated respiratory pathogens,⁴ we hypothesized that it could be used to detect exhaled vola-

tiles from the breath of patients with ventilator-associated pneumonia (VAP) and that the response pattern could be correlated to a clinical pneumonia score.^{5,6}

Materials and Methods

Over a period of 4 months, 400 patients from a 52-bed surgical intensive care unit at the Hospital of the University of Pennsylvania were screened for study eligibility as defined below. This number was selected based on the assumption that 10% of the screened patients would be sampled, and a power analysis using an alpha of 0.05 and a P of 0.8. We screen all mechanically ventilated patients for VAP as defined by Centers for Disease Control criteria.⁷ With approval from the Institutional Review Board (which waived the need for consent), samples of exhaled breath were acquired from 38 randomly selected, mechanically ventilated patients and analyzed using a commercially available electronic nose (Cyrano Sciences, Pasadena, Ca).

Patients undergoing positive pressure ventilation *via* endotracheal tube were eligible. Patients with respiratory distress, 10 cm H₂O or greater of positive end-expiratory pressure, high levels of pressure support (>20 cm H₂O), or respiratory rate >35 breaths/min were excluded. These exclusion criteria were designed to preclude sampling on patients with a significant degree of respiratory insufficiency, as the acquisition of six breath samples takes 30–45 min and patients with this degree of mechanical ventilatory support often require interventions that would interfere with uninterrupted data acquisition. In addition, flow-triggering was discontinued during sampling to maximize signal to noise ratio, and we therefore chose to restrict sampling to patients who were on relatively minimal ventilatory support.

All patients were given a Clinical Pulmonary Infection Score (CPIS) based on the one initially described by Pugin et al.⁸ and later modified by Luna *et al.*⁹ All patients were mechanically ventilated with positive pressure ventilation. For the period of sample acquisition, “flow by” (used either as a triggering adjunct or primary mode) on the patient ventilator was suspended and the heat and moisture exchanger was removed (if present). Breath samples were obtained from an access port in the expiratory limb of the ventilator circuit just distal to the Y piece attached to the patient’s endotracheal tube. The electronic nose was fitted with an Acrodisc (Pall, East Hills, NY) CR 25 mm syringe filter with a polytetrafluoroethylene membrane. Approximately 30 ml of expired air were sampled over 30 s. Patients were sampled six times

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Received from the Department of Anesthesia, University of Pennsylvania, Philadelphia, Pennsylvania. Submitted for publication May 5, 2004. Accepted for publication October 1, 2004. Supported, in part, by Smiths Detection, Inc., Pasadena, California, to fund the salary of a research nurse.

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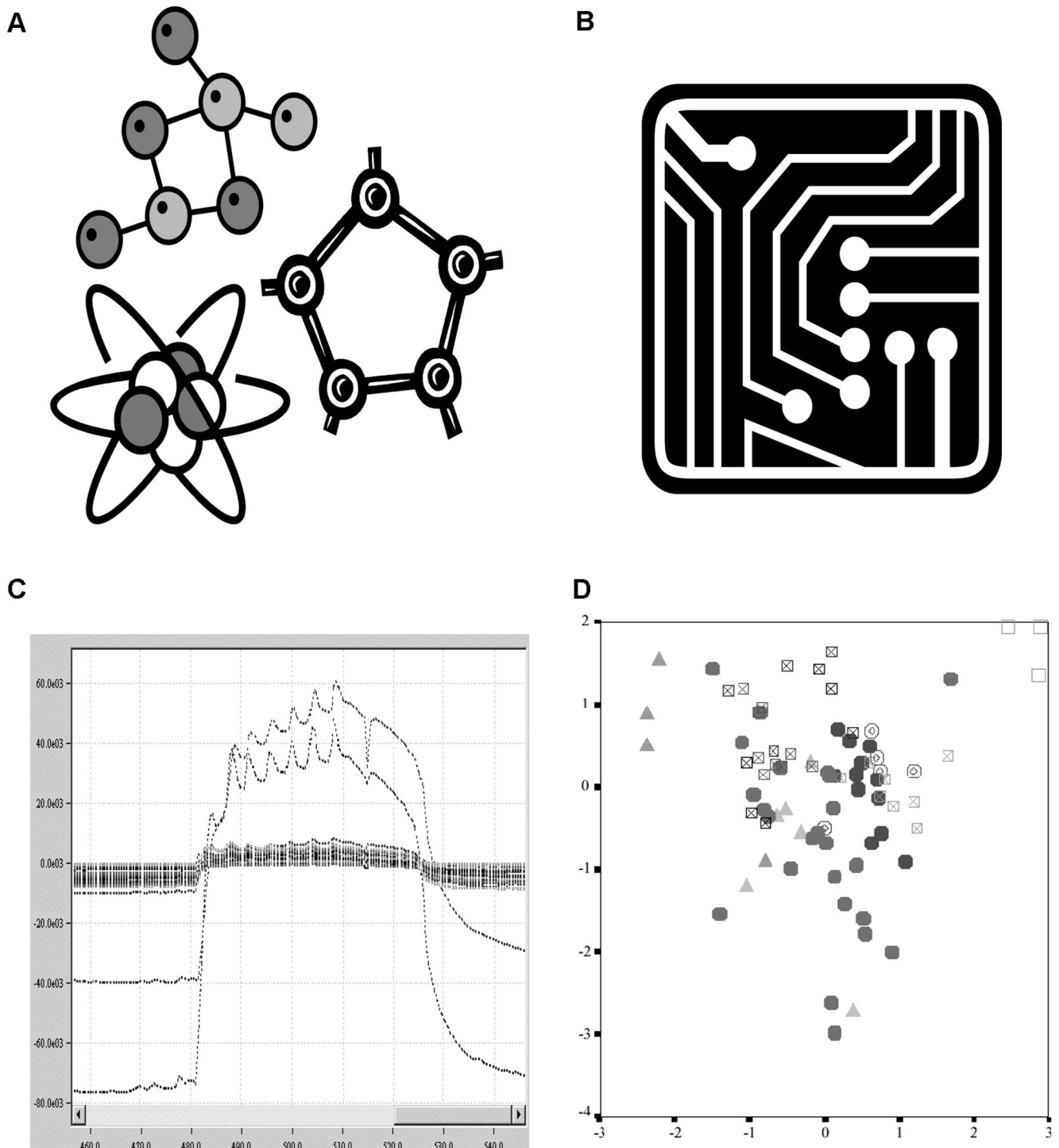


Fig. 1. Electronic nose analysis: the various molecules in an odor (A) interact with the chip based e-nose sensor array (B). The resistance of each of the sensors on the chip changes (*y axis*) over time (*x axis*) as they interact with the odorant molecules (C), and the aggregate response of the sensors is used to analyze and map (as shown in D) individual odors, after the application of a dimensionality reduction technique such as principal components analysis.

consecutively in a period of 30–45 min using the same procedure. The sample information was downloaded from the electronic nose to a computer for analysis.

The electronic nose used for this study is handheld. It includes a small pump, a battery power source, micro-circuitry, and a sensor array. Each sensor in the 32-sensor

array consists of a carbon black/polymer composite, in which the carbon black creates conducting pathways through the polymer. As the specific polymer composite interacts with the molecules in the sample volume, it swells, disrupting the carbon black conducting pathways to a variable degree and thereby altering the resis-

tance of the sensor. Carbon black/polymer composite sensors are typically able to detect at a level of <0.1% of saturated vapor pressure (ppm to ppb).

Statistical Analysis

The data were analyzed using linear partial least squares regression and nonlinear partial least squares regression (PLS toolbox for Matlab, Eigenvector Research Inc, Manson, WA) for constructing predictive models relating response variables to explanatory variables.¹⁰⁻¹² Partial least squares was originally developed for econometrics but is now used in chemometrics and industrial applications in which multivariate data are used to classify or predict outcomes or responses. This method of analysis is directly applicable and frequently used in chemometrics for the analysis of odors using an electronic nose, in which multivariate (from multiple vapor sensors within the e-nose), potentially colinear (because some of the sensors may track together) data are acquired and correlated with outcomes (*i.e.*, known odors or, in this case, known scores). Multiple linear regression is not appropriate for this analysis because the relationship between the predictive factors and the outcomes is not well understood and because the predictors have the potential to be interrelated (colinear). The nonlinear partial least squares method is a nonlinear classification technique that extends the partial least squares approach and remedies some of its shortcomings.

Partial least squares regression was used to relate CPIS scores to scores predicted by the e-nose and construct a Bland-Altman plot. Nonlinear partial least squares regression was used to determine the degree of correlation between the e-nose and CPIS scores. Cross-validation was used for both the partial least squares and nonlinear partial least squares CPIS prediction models to prevent over-fitting of the data. In cross-validation, the model relating actual outcomes to predicted outcomes is developed for all the pairs (of actual and predicted data) save one and then tested on that holdout case. This is repeated *n* times, with each case used as the validation case in turn.

Results

Demographic information about the study patients are listed in table 1. The bacterial species cultured (using semiquantitative cultures) from tracheal secretions of patients with CPIS scores greater than 5 are listed in table 2. Culture data were recorded if a sample was acquired within 24 h of breath sampling, and therefore available in 12 of 19 patients with a CPIS score greater than 5. The actual CPIS scores in the patient population were normally distributed. The data generated using nonlinear partial least squares regression is plotted in

Table 1. Patient Demographics

Parameter	CPIS score <6	CPIS score ≥6
Age (mean ± SD)	62.5 ± 4.3	63.5 ± 3.1
Sex, n (%)		
Male	12 (64)	12 (64)
Female	7 (36)	7 (36)
Cardiac surgery	3	2
Abdominal surgery	4	5
Major vascular	4	4
Neurosurgery	5	6
Trauma	3	2

CPIS = clinical pulmonary infection score.

figure 2. Each point represents the mean score for the six samples (\pm SEM) acquired during an individual sampling session. The plot shows the correlation between the pneumonia score and the score predicted by the electronic nose. Figure 3 shows a Bland-Altman plot of the data and indicates a proportional deviation between the two measurements, which is also evident in figure 2.

Discussion

Recent studies using gas chromatography and mass spectrometry have characterized some of the volatiles excreted from the body. Maple syrup urine disease, for example, is identifiable by a sweet aroma in the urine, which has been analyzed and determined to be attributable to sotolone.¹³ The breath of schizophrenics has been found to contain volatile organic compounds including pentane and carbon disulfide.^{14,15} Breath analysis has also been used as a noninvasive diagnostic test to detect *Campylobacter pylori* in the stomach.¹⁶ Pathologic processes in the distal gut have been diagnosed by breath analysis.¹⁷⁻¹⁹ Various breath tests have been used to evaluate hepatic function.²⁰⁻²² Vapor pressure has been used as a method of measuring sweat osmolality in screening for cystic fibrosis.²³ There is a substantial literature regarding breath analysis in the occupational health field.²⁴⁻³² Finally, previous research has also shown alterations in the pentane/isoprene ratio in the

Table 2. Tracheal culture results

Bacteria	Number of patients
<i>Pseudomonas aeruginosa</i>	2
<i>Candida albicans</i>	2
<i>Staphylococcus aureus</i>	1
<i>Citrobacter koseri</i>	1
Group C <i>Streptococcus</i>	1
Group B <i>Streptococcus</i>	1
<i>Escherichia coli</i>	2
<i>Acinetobacter baumannii</i>	1
<i>Proteus mirabilis</i>	1

Organisms cultured from sputum of patients with within 24 hours of e-nose sampling where clinical pulmonary infection score ≥ 6 (semiquantitative cultures).

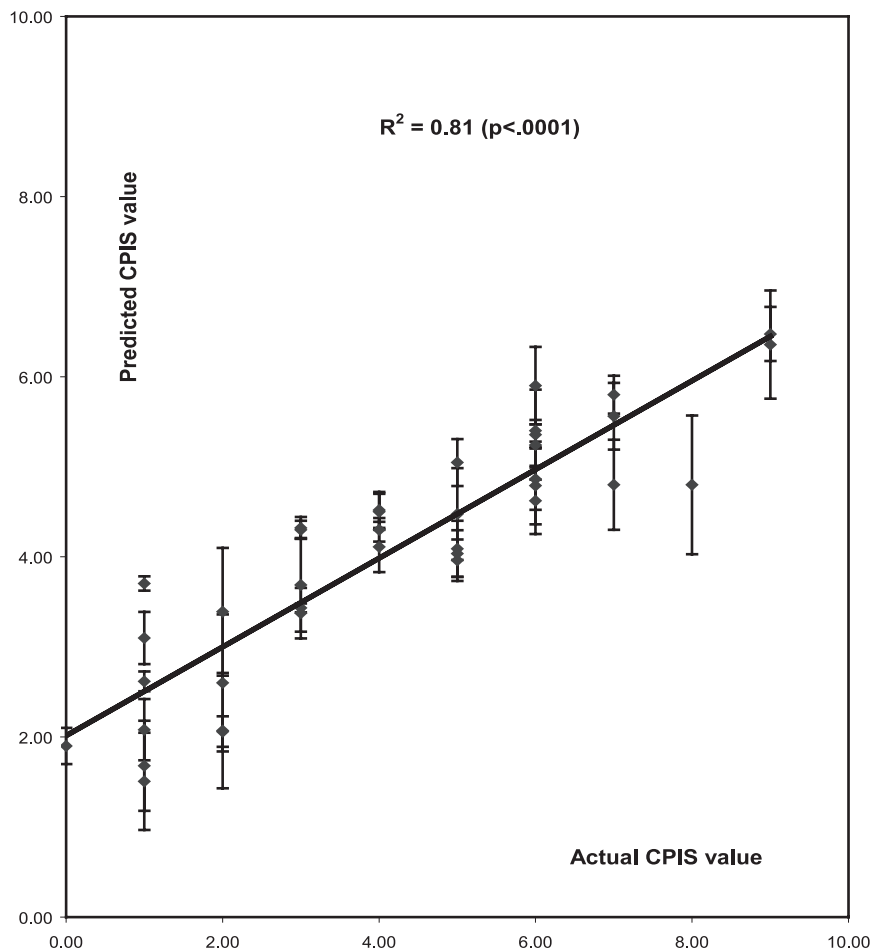


Fig. 2. Nonlinear partial least squares regression of e-nose predicted *versus* actual clinical pneumonia infection score (CPIS) value.

expired gas of critically ill patients with adult respiratory distress syndrome and pulmonary infection.^{33,34}

Although exhaled or expressed volatiles contain information pertaining to pathophysiologic processes,³⁵⁻³⁷ gas chromatography and mass spectroscopy are difficult

to bring to the bedside. There are a variety of other sensor technologies in current generation electronic noses.³⁸⁻⁴⁰ Some of them are commercially available and deployed, whereas others are being developed. They vary in their sensitivity and response to different odors.

The initial application of an electronic nose to the analysis of exhaled gases in intensive care patients was done using an e-nose consisting of an array of 32 intrinsically conducting polymer sensors. In this study, we used an e-nose with a sensor array consisting of the carbon black/polymer composites described above. There are several advantages to the use of this sensor technology. The first is that it is handheld, whereas that of the conducting polymer array e-nose is table-mounted (as are most of the other commercially available e-noses). The smaller footprint of the handheld device makes direct aspiration of exhaled gas into the e-nose possible, thereby obviating problems associated with transport of a gas sample from the bedside to the device. This type of sensor is stable and performs better in the presence of water vapor than the intrinsically conducting polymer e-nose array, permitting more reliable performance in the analysis of humid samples, such as expired gas from mechanically ventilated patients.

VAP occurs in 10-25% of patients ventilated for longer

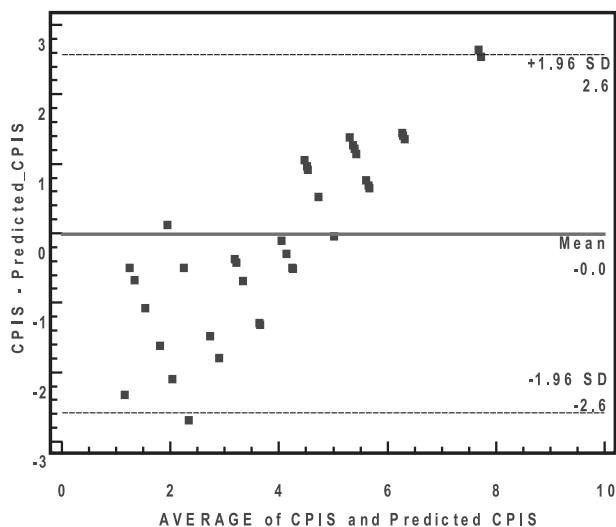


Fig. 3. Bland-Altman plot showing a proportional deviation between the two measurements CPIS = clinical pneumonia infection score.

than 48 h, and it is the most commonly acquired infection in intensive care patients according to some studies.⁴¹ Most studies show an association between VAP and increased mortality. One commonly employed approach to the clinical diagnosis of VAP is based on a "pneumonia score," with or without invasive procedures such as bronchoscopy.^{42,43}

Chemometrics is the field of extracting information from multivariate chemical data as in odor analysis. Patterns of association can exist in data sets, but relationships among samples can be difficult to discover when the data matrix exceeds three or more features. Exploratory data analysis can reveal hidden patterns in complex data by reducing the information to a more comprehensible form. It can thereby reveal outliers and indicate whether there are patterns or trends in the data. Algorithms such as principal component analysis are designed to reduce large complex data sets into a series of optimized and interpretable views. These views emphasize the natural groupings in the data and show which variables most strongly influence those patterns.

The goal of chemometric regression analysis, the statistical approach used in this work, is to develop a calibration model that correlates information in a set of known measurements (the e-nose sensor responses) to a standard (the CPIS score). Commonly used algorithms for performing chemometric calibration include partial least squares and nonlinear partial least squares regression, and they are designed to eliminate problems associated with noise and colinear sensor responses. Because these regression algorithms are based in factor analysis, the entire group of known measurements is considered simultaneously, and information about correlations among the variables is automatically built into the calibration model.

Methodological Limitations

There are a number of weaknesses in this study that preclude the immediate adoption of e-nose analysis as a diagnostic approach for VAP. The e-nose provides an aggregate response of the sensors to the chemical analytes, but we have not determined what those analytes are or whether they bear any relationship to the disease under study. The e-nose may smell bacterial metabolites, gases emitted during the bacteriocidal immune response, or a combination thereof. The specific molecules responsible for the sensor response pattern were not analyzed. It is clearly desirable to better characterize the molecular signals using gas chromatography and mass spectroscopy, both to enhance our understanding of the exhaled volatiles in lung infection and to develop a sensor array tuned to those volatiles. It is also unclear whether the "smell" of nonpathologic bacterial colonization of the trachea is the same as that of true VAP.

The design of this study only permits the conclusion that the e-nose sensor responses correlate with the CPIS

score. Further studies, in which a "trained" or calibrated e-nose is used to prospectively assign a CPIS score (which is then compared to the actual CPIS), will be necessary to determine whether the e-nose can be used in a clinical setting as a diagnostic adjunct. Additional studies will be necessary to determine whether the e-nose can track bacterial load (*i.e.*, the quantity of bacteria derived from a culture) or type.

Conclusion

The breath of mechanically ventilated patients is well suited to diagnostic analysis, and this data suggests that an electronic nose may be capable of distinguishing between the infected and uninfected states. The demonstration of a correlation between a clinical pneumonia score and a pneumonia score based on the e-nose's sensor response provides the possibility that a sufficiently sensitive and specific e-nose could be used as a univariate, noninvasive, concurrent measurement for the diagnosis of VAP. Although the CPIS score has not been shown to be diagnostic of VAP, it has been shown to be sensitive,⁴⁴ and it is conceivable that a high predicted CPIS score from the e-nose could be used as a trigger for noninvasive (tracheal aspirate) or invasive (bronchoscopic) diagnostic studies. One can imagine, for example, the use of a probability of infection score derived from e-nose monitoring in the management of critically ill mechanically ventilated patients. Further study will be needed to determine whether the electronic nose can be used to detect the onset of VAP, follow its response to treatment, or distinguish among infectious etiologies.

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