Volatile organic compounds in the breath of patients with schizophrenia

M Phillips, G A Erickson, M Sabas, J P Smith, J Greenberg

Abstract

Aims—To analyse the breath of patients with schizophrenia for the presence of abnormal volatile organic compounds.

Methods—A case comparison study was performed in two community hospitals in Staten Island, New York. Twenty-five patients with schizophrenia, 26 patients with other psychiatric disorders, and 38 normal controls were studied. Alveolar breath samples were collected from all participants, and volatile organic compounds in the breath were assayed by gas chromatography with mass spectroscopy. Differences in the distribution of volatile organic compounds between the three groups were compared by computerised pattern recognition analysis.

Results—Forty-eight different volatile organic compounds were observed in the breath samples. Three separate pattern recognition methods indicated an increased differentiation capability between the patients with schizophrenia and the other subjects. Pattern recognition category classification models using 11 of these volatile organic compounds identified the patients with schizophrenia with a sensitivity of 80-0% and a specificity of 61-9%. Volatile organic compounds in breath were not significantly affected by drug therapy, age, sex, smoking, diet, or race.

Conclusions—Microanalysis of volatile organic compounds in breath combined with pattern recognition analysis of data may provide a new approach to the diagnosis and understanding of schizophrenia. The physiological basis of these findings is still speculative.

(J Clin Pathol 1995;48:466–469)

Keywords: Breath tests, schizophrenia, pattern recognition

Patients with schizophrenia have been reported to give off a persistent unpleasant smell which is not related to uncleanliness.12 Several other diseases have also been associated with a distinctive aroma, such as the fetor hepaticus of liver failure, the smell of decomposing apples in diabetic acidosis, and the odour of fresh baked brown bread in typhoid fever. The common feature of these disorders might be an abnormal metabolic pathway which manufactures a volatile organic compound with a pungent smell.

Attempts to identify the responsible compound in the sweat of patients with schizophrenia have not met with success; however, abnormal body odours can now be studied by analysis of the volatile organic compounds in the breath. Microanalysis of alveolar breath opens a unique and non-invasive window onto the pool of volatile compounds which circulate in the blood and rapidly diffuse across the pulmonary alveolar membrane.13 Gas chromatography combined with mass spectroscopy (GC–MS) has revealed more than 100 volatile organic compounds in normal human breath, many of them in picomolar (10−12 M) concentrations.14 Breath tests have shown that patients with schizophrenia expire increased amounts of pentane and carbon disulphide.8–10

Since GC–MS analysis reveals so many different compounds in a single sample of alveolar breath, the search for a disease marker is a difficult undertaking. Using techniques for statistical comparison volatile organic compounds can be tested one at a time for distribution differences between different populations. However, in the absence of an obvious major concentration difference in one or more of these compounds, computerised pattern recognition analysis can provide a powerful technique for identifying small yet significant changes in several volatile compounds simultaneously.11,12

We report here a study in which microanalysis of volatile organic compounds in the breath was combined with pattern recognition analysis in an attempt to detect abnormalities in multiple volatile compounds in patients with schizophrenia.

Methods

COLLECTION AND ASSAY OF VOLATILE ORGANIC COMPOUNDS

The method has been described.13 Using a mobile apparatus, alveolar breath was collected from a donor inspiring chemically purified air. The volatile organic compounds in 10 litres of breath were trapped by adsorption to activated carbon and molecular sieve. The volatile organic compounds were thermally eluted from the trap in a microprocessor controlled automatic desorber, concentrated by two stage cryofocusing, then assayed by GC–MS with an ion trap detector. Each compound was identified by its mass spectrum and quantified by the area under the curve of its chromatographic peak.

HUMAN SUBJECTS

The subjects and recruitment procedures employed in this study have been reported pre-
PATTERN RECOGNITION ANALYSIS OF DATA

Multivariate pattern recognition data analysis was performed using the Pirouette software system (Infometrix). Volatile organic compound concentrations in alveolar breath and background air were analysed separately. Hierarchical clustering associations were investigated among subjects in the same group, using complete link, single link, and incremental link methods. Scatter plots of the case scores for several principal components were examined for indications of structure (group associations) and outliers (highly distinct cases). Classification accuracies and diagnostics for predicting each case against its known health/disease status were assessed using K-Nearest Neighbor (KNN) and Soft Independent Modeling of Class Analogy (SIMCA) methods.\textsuperscript{11,12}

VALIDATION OF PATTERN RECOGNITION ANALYSIS

The validity of the multivariate model was tested by evaluating its predictive capability for cases not included in the development of the model. The KNN results, listed above, were calculated in a "leave one out" method, where each sample was classified according to the neighbour votes of all the remaining samples. The SIMCA models were tested with a one third:two thirds cross validation method, where the models were retrained three times, each time leaving out one third of the cases and using the remaining two thirds for model development. The models were then applied to the excluded one third to judge prospective classification capability.

Results

HUMAN SUBJECTS

Breath samples were obtained from all subjects without any adverse effects. Multiple correlation tests did not show any statistically significant differences between the three groups in the distribution of age, sex, tobacco use, or race, nor was there any significant difference in the use of neuroleptic drugs between the patients with schizophrenia and the psychiatric controls.\textsuperscript{15}

PATTERN RECOGNITION ANALYSIS OF VOLATILE ORGANIC COMPOUNDS

Multivariate data analysis provided three separate indications that the patterns of breath volatiles were distinctly different in patients with schizophrenia. Cluster analysis showed an imbalanced distribution on the dendrogram branches, while both a 2-D principal component scatter plot and a SIMCA category classification model separated most of the patients with schizophrenia from the other subjects. In contrast, results from the analysis of inspired air did not show an imbalance or differentiation between the three groups, indicating that the experimental design did not introduce artefacts arising from differences in environmental air exposure.

Figure 1 Hierarchical cluster analysis dendrogram of normalised alveolar breath data using the incremental link method. Five branches are defined at a similarity value of 0.40 and are numbered on the dendrogram starting at the top. Normal cases are highlighted by black squares attached to the branch end at similarity 1.0. Normals are more heavily distributed on branch 3 and portions of branch 5.
Hierarchical cluster analysis

Table 1  Hierarchical cluster analysis branch case distribution. Group percentages in parentheses

<table>
<thead>
<tr>
<th>Branch</th>
<th>Normal</th>
<th>Psychiatric controls</th>
<th>Schizophrenia patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (n=5)</td>
<td>1 (20%)</td>
<td>1 (20%)</td>
<td>3 (60%)</td>
</tr>
<tr>
<td>2 (n=28)</td>
<td>9 (32%)</td>
<td>11 (39%)</td>
<td>8 (29%)</td>
</tr>
<tr>
<td>3 (n=11)</td>
<td>8 (73%)</td>
<td>1 (9%)</td>
<td>2 (18%)</td>
</tr>
<tr>
<td>4 (n=7)</td>
<td>0 (0%)</td>
<td>4 (57%)</td>
<td>3 (43%)</td>
</tr>
<tr>
<td>5 (n=37)</td>
<td>19 (51%)</td>
<td>9 (24%)</td>
<td>9 (24%)</td>
</tr>
<tr>
<td>Total cases</td>
<td>38</td>
<td>26</td>
<td>25</td>
</tr>
</tbody>
</table>

The dendrogram (fig 1) is cut into five branches by a dotted vertical line at similarity value 0.40. Normal subjects are highlighted by a square attached to the end of the subject's individual branch below the similarity value of 1.0. The distribution of normals and patients with schizophrenia was highly imbalanced: normals were more heavily distributed on branch 3 and portions of branches 2 and 5 (branches numbered at the cut from the top) while the patients with schizophrenia were more heavily distributed on branches 1, 2, and 4. The distribution of cases is detailed in table 1.

2-D principal component (pc) scatter plot—A scatter plot of the case scores for pc4 vs pc2 is shown in fig 2. A hand drawn discriminant line in the plot separated 39/63 of non-schizophrenic patients from 20/25 of patients with schizophrenia (that is, sensitivity = 80-0%; specificity = 61-9%). The variables which contributed most influentially to the two principal components are shown in table 2, with their loading coefficients.

Category classification—The discriminating separation between subjects with and without schizophrenia (fig 2) was substantiated by the complementary approach of group differentiation using the two methods in Pirouette, KNN, and SIMCA. SIMCA results yielded the best separation. Using all of the 46 volatile organic compounds, SIMCA yielded a sensitivity of 40% and a specificity of 97%. When 11 compounds were used, chosen from the highest contributors to principal components in fig 2 and SIMCA models, sensitivity increased to 68-0% (17/25), while specificity decreased to 84-1% (53/63). These 11 compounds are shown in table 2 with their SIMCA modelling power coefficients and principal components loading.

Discussion

Microanalysis of the volatile organic compounds in alveolar breath combined with pattern recognition analysis identified patients with schizophrenia with a sensitivity of 80-0% and a specificity of 61-9%. Since, as Leff has observed, “The history of research into schizophrenia is replete with ‘breakthroughs’ which have later turned out to be illusory” 15 these findings merit sceptical scrutiny for possible sources of error.

Did all of the subjects designated as patients with schizophrenia really suffer from the dis-

Table 2  SIMCA modelling power and loadings for volatile organic compounds in alveolar breath and inspired air

<table>
<thead>
<tr>
<th>Volatile organic compound</th>
<th>SIMCA modelling power</th>
<th>Loading</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-Methylbutane</td>
<td>A</td>
<td>0.1979</td>
</tr>
<tr>
<td>B</td>
<td>0.2300</td>
<td>0.2529</td>
</tr>
<tr>
<td>Trichlorofluoromethane</td>
<td>A</td>
<td>0.834</td>
</tr>
<tr>
<td>B</td>
<td>0.2637</td>
<td>0.5044</td>
</tr>
<tr>
<td>1-Pentanol</td>
<td>A</td>
<td>0.754</td>
</tr>
<tr>
<td>B</td>
<td>0.2707</td>
<td>0.4916</td>
</tr>
<tr>
<td>Pentane</td>
<td>A</td>
<td>0.928</td>
</tr>
<tr>
<td>B</td>
<td>0.2028</td>
<td>0.1904</td>
</tr>
<tr>
<td>Benzene</td>
<td>A</td>
<td>0.819</td>
</tr>
<tr>
<td>B</td>
<td>0.3930</td>
<td>0.3851</td>
</tr>
<tr>
<td>1-Chloro-2-methylbutane</td>
<td>A</td>
<td>0.743</td>
</tr>
<tr>
<td>B</td>
<td>0.2855</td>
<td>0.2011</td>
</tr>
<tr>
<td>2,3,3-Trimethylpentane</td>
<td>A</td>
<td>0.919</td>
</tr>
<tr>
<td>B</td>
<td>0.5874</td>
<td>0.1369</td>
</tr>
<tr>
<td>2,2-Dimethylbutane</td>
<td>A</td>
<td>0.724</td>
</tr>
<tr>
<td>B</td>
<td>0.3432</td>
<td>0.3217</td>
</tr>
</tbody>
</table>

Class 1 = normal; class 2 = psychiatric controls; class 3 = schizophrenic.
Breath volatiles in schizophrenia

breath of schizophrenia patients has been observed with drug treatment. This gastroesophageal reflux might also cause an accumulation of oxygen free radicals, which could contribute to the increased levels of volatile compounds.

We conclude that microanalysis of volatile organic compounds in alveolar breath, combined with pattern recognition analysis, might allow us to distinguish patients with schizophrenia from non-schizophrenic controls. Further studies are required to validate these findings and to optimise breath testing methodology. With improved sensitivity and specificity, microanalysis of volatile organic compounds in the breath might offer a new approach to the detection of schizophrenia and a way to better understand the metabolic basis of the disease.

Volatile organic compounds in the breath of patients with schizophrenia.

M Phillips, G A Erickson, M Sabas, J P Smith and J Greenberg

*J Clin Pathol* 1995 48: 466-469
doi: 10.1136/jcp.48.5.466

Updated information and services can be found at:
http://jcp.bmj.com/content/48/5/466

**Email alerting service**

Receive free email alerts when new articles cite this article. Sign up in the box at the top right corner of the online article.

**Notes**