Volatile organic compounds in the breath of patients with schizophrenia

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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)

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Accepted for publication 26 September 1994

Patients with schizophrenia have been reported to give off a persistent unpleasant smell which is not related to uncleanliness.[1][2] 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.[1] 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[3]; 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.[4][5] 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.[6][7] 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 chromatograph peak.

HUMAN SUBJECTS

The subjects and recruitment procedures employed in this study have been reported pre-

viously.[10] In summary, 88 volunteers were studied at St Vincent's Medical Center of Richmond and Bayley Seton Hospital in Staten Island, New York. All gave their informed consent to participate in this research, which was approved by the institutional review boards of both institutions. They comprised:

(1) *Normal controls*—A group of 37 normal volunteers was drawn from the medical and nursing staff of both institutions. None gave any history of psychiatric illness.

(2) *Psychiatric controls*—Twenty six subjects were recruited from the psychiatric inpatient units of both hospitals. This was an intentionally heterogeneous group, selected in order to provide a diverse spectrum of non-schizophrenic psychiatric disorders. All had been diagnosed as suffering from a psychiatric illness other than schizophrenia by DSM-III-R criteria.[14]

(3) *Patients with schizophrenia*—Twenty five subjects were studied who had been previously diagnosed as suffering from schizophrenia. All had fulfilled DSM-III-R criteria for the diagnosis of schizophrenia before the hospital admission when the study was performed. All had experienced a recent exacerbation requiring admission to a psychiatric inpatient unit, where they shared the same environment and diet as the non-schizophrenic psychiatric controls.

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.[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.[10]

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.
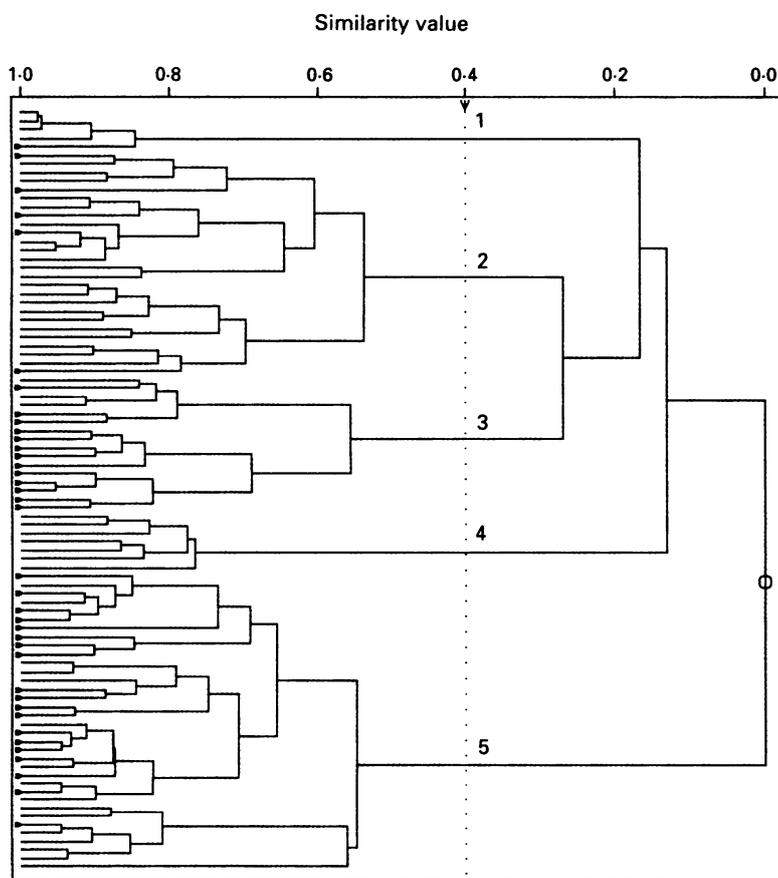
Similarity value

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.

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

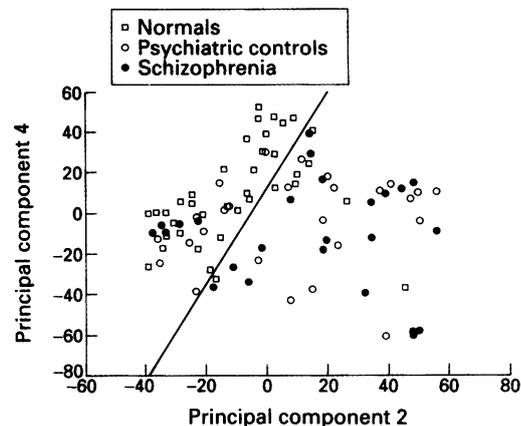|	Normals	Psychiatric controls	Schizophrenia patients
Branch 1 (n = 5)	1 (20%)	1 (20%)	3 (60%)
Branch 2 (n = 28)	9 (32%)	11 39%)	8 29%)
Branch 3 (n = 11)	8 (73%)	1 (9%)	2 (18%)
Branch 4 (n = 7)	0 (0%)	4 (57%)	3 (43%)
Branch 5 (n = 37)	19 (51%)	9 (24%)	9 (24%)
Total cases	38	26	25

Figure 2   Alveolar breath data: principal component analysis. The scores for PC axis 4 are shown plotted versus PC axis 2. A discriminant function corresponding to the diagonal line would distinguish patients with schizophrenia with a sensitivity of 80·0% and a specificity of 61·9%.

Hierarchical cluster analysis—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 *v* 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

Volatile organic compound		SIMCA modelling power			Loading			
		Class 1	Class 2	Class 3	PC1	PC2	PC3	PC4
2-Methylbutane	B				0·1979	0·1913	0·4152	−0·1037
	A				0·2380	0·2529	0·4082	−0·1507
Trichlorofluoromethane	B		0·834					
	A							
2-Pentanol	B	0·775	0·891	0·955	0·2637	0·5044	−0·4552	−0·6478
	A				0·2707	−0·9106	0·2356	
Pentane	B		0·754	0·761	0·1704	0·4997	0·4400	0·1378
	A						0·2154	0·2236
Dichloromethane	B							
	A				0·1294		0·2000	
Trichloroethene	B	0·928		0·804	0·2218	−0·2011		0·4422
	A				0·2028	0·1904		0·4729
Benzene	B	0·819	0·782	0·708	0·3930	−0·3851		0·2753
	A				0·4049	−0·5607		
1-Chloro-2-methylbutane	B	0·743			0·2800			0·1411
	A				0·2855	0·1651		0·2363
2,3,3-Trimethylpentane	B	0·919	0·910	0·934	0·5636	−0·5973	0·2554	−0·3911
	A				0·5874	0·1369	0·2084	−0·6185
2,2-Dimethylbutane	B		0·724		0·1793			
	A				0·2283	−0·3617		
Tetrachloroethene	B	0·710			0·3424	−0·1937		0·2898
	A				0·3217	−0·2739		0·2274

Class 1 = normal; class 2 = psychiatric controls; class 3 = schizophrenic.

ease? All had been admitted to the hospital with an episode consistent with an acute exacerbation of schizophrenia, and had previously been diagnosed as suffering from schizophrenia according to DSM-III-R criteria. These stringent criteria include continuous signs of the disturbance for at least six months. It is unlikely that any patients not suffering from schizophrenia could have been included in this group in error. It is equally unlikely that any of the normals or the psychiatric controls were suffering from schizophrenia.

Could the findings have been skewed by other factors, such as treatment with neuroleptic drugs? All of the patients with schizophrenia had been treated with neuroleptic drugs, unlike approximately half of the psychiatric controls and none of the normals. However, drug therapy did not appear to have skewed the composition of breath volatile organic compounds since pattern recognition analysis did not separate the psychiatric controls who had received neuroleptics from either the untreated psychiatric controls or the normal subjects. Other possible confounding variables such as age, sex, race, and cigarette smoking showed no statistically significant differences between the three groups.[10]

Could the separation observed with pattern recognition analysis have arisen from overfitting the data, some of which may have arisen from instrumental measurement error and random noise? A potential hazard of multivariate modelling is the tendency to overfit the category classifications by using too many variables or principal components in the models. This may introduce apparent differences between the classes, which are in fact due to random noise. However, the results of validation testing suggest that this did not occur: the principal component models using two to three components retained predictive capability, and their predictive performance only began to be degraded when more than five principal components were used. In addition, three philosophically different mathematical methods separated the patients with schizophrenia from the other subjects, supporting the conclusion that these differences were real rather than artefactual.

What physiological abnormalities might have accounted for the distinctive pattern of volatile organic compounds in the breath of the patients with schizophrenia? Among the most influential compounds listed in table 2, pentane has previously been observed in increased amounts in the breath of patients with schizophrenia,[8–10] possibly because of an accumulation of oxygen free radicals causing accelerated peroxidation of membrane lipids.[16] The compounds listed in table 2 may indicate derangements in other biochemical pathways, but their source is still unknown.

Could these findings account for the unusual smell of patients with schizophrenia, which prompted these studies in the first place? Again, the answer is not yet known, though the question suggests a future research study designed to compare the odour of patients with schizophrenia to the odour of a synthetic mixture of the compounds listed in table 2.

Was the full range of biochemical variation in all three groups adequately represented in this study? Only 25 patients with schizophrenia were studied, so it is possible that we failed to observe subcategories of schizophrenia with different biochemical profiles. This has important inferences for pattern recognition analysis, since SIMCA classification accuracy would most probably improve if schizophrenia were modelled by more than one class of biochemical variant.

We conclude that microanalysis of volatile organic compounds in alveolar breath, combined with pattern recognition analysis, appeared 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 better understanding of the metabolic basis of the disease.

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