
PILOT STUDY: VOLATILE ORGANIC COMPOUNDS AS A DIAGNOSTIC MARKER FOR HEAD AND NECK TUMORS

Joachim Schmutzhard, MD,¹ Josef Rieder, MD,² Martina Deibl, PhD,³ Ilona M. Schwentner, MD,¹ Stefan Schmid, MD,² Philip Lirk, MD,² Irene Abraham, MD,¹ Andreas R. Gunkel, MD¹

¹ Department of Otolaryngology, Innsbruck Medical University, Innsbruck, Austria.

E-mail: joachim.schmutzhard@i-med.ac.at

² Department of Anesthesiology and Critical Care Medicine, Innsbruck Medical University, Innsbruck, Austria

³ Department of Biostatistics and Documentation, Innsbruck Medical University, Innsbruck, Austria

Accepted 25 September 2007

Published online 19 February 2008 in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/hed.20779

Abstract: *Background.* In the last decade, the analysis of volatile organic compounds (VOC) has undergone a rapid development. In this pilot study, patients with HNSCC were tested with a proton transfer reaction-mass spectrometry in order to establish a minimal invasive screening method.

Methods. Overall in a period of 2 years, 22 carcinoma patients were recruited for the study. All patients had a newly diagnosed histologically secured squamous cell carcinoma of the upper aerodigestive tract. These results were statistically compared with 3 control groups: healthy controls, high-risk, and posttherapy patients.

Results. Two hundred nine different masses were measured; 188 of these were evaluated. The statistical workup of the 4 study groups produced 42 different masses, which showed a statistically significant difference from the carcinoma group compared with the control groups.

Conclusion. A screening method for HNSCC using VOC seems to be possible, but further investigation is necessary. ©2008 Wiley Periodicals, Inc. *Head Neck* 30: 743–749, 2008

Keywords: proton transfer reaction-mass spectrometry; HNSCC; VOC-profile; screening method; noninvasive

Correspondence to: J. Schmutzhard

Contract grant sponsor: the Austrian Science Fund (FWF); contract grant number: P-14149MED; Contract grant sponsors: the Innovationspreis der Tiroler Sparkasse and the Daniel Swarovski research award of the Medical Faculty, University of Innsbruck.

© 2008 Wiley Periodicals, Inc.

Over the past decade, the analysis of volatile organic compounds (VOC) has seen an enormous boost. Different studies dealing with this topic demonstrated that various diseases (eg, lung cancer,¹ breast cancer,² schizophrenia,³ and recent smoking behaviors⁴) are associated with specific VOC profiles in human exhaled air. These profiles differ explicitly from that of healthy subjects and are specific for the mentioned diseases.

Phillips et al showed the diagnostic capacity of special VOC profiles for lung and breast cancer.^{1,2} These VOC profiles are thought to originate from the different metabolic pathways that are found in malignant cell populations.⁵ Therefore, special VOC profiles should be found in different types of malignant diseases.

In this study, we tried to extract a special VOC profile for malignant head and neck tumors, especially the squamous cell carcinoma of this region. The exhaled air of patients with head and neck carcinomas was analyzed with a proton transfer reaction-mass spectrometry (PTR-MS) and compared with a healthy control group; a group of people with a higher-risk profile—called the high-

risk group; and a group of patients after successful therapy—called the posttherapy group.

PATIENTS AND METHODS

Selection of Patients. The study was approved by the local ethics committee, and a written informed consent was obtained from each participant. The patients were recruited and tested at the ENT-department of the Medical University of Innsbruck. Inclusion criteria for the carcinoma group were a squamous cell carcinoma of the upper aerodigestive tract, which was histologically confirmed, but still untreated. Exclusion criteria were a radiotherapy or chemotherapy before the testing and another diagnosed malignant carcinoma at the time of testing.

The healthy control group consisted of volunteers. Inclusion criteria for the healthy control group were at least 40 years of age, no malignant disease, and a negative nicotine and alcohol history.

People with chronic laryngitis and a positive alcohol and nicotine history qualified for the high-risk group. Similar to the posttherapy group, the high-risk groups were volunteers from the outpatients of the ENT department. The inclusion criteria for the posttherapy group were a relapse-free survival for at least 6 months after successful therapy.

Overall, in a period of 2 years, 22 carcinoma patients were recruited for the study. All patients had a newly diagnosed histologically confirmed squamous cell carcinoma of the upper aerodigestive tract.

Analysis. Informed consent was obtained from each participating patient. Afterward a short questionnaire concerning personal habits, such as drinking and smoking, was answered and an appointment immediately preoperatively was arranged to collect the sample. In our institution, every patient undergoes a panendoscopy under general anesthesia as a staging measurement. This point of time was selected, because the sample should be given after fasting, to keep environmental influences to a minimum.

The test person's breath was collected with special 3-liter Teflon-bags (Adtech, Gloucestershire, UK). Each participant was asked to fill 3 of the Teflon-bags with 1 single deep breath. The Teflon-bags are known to be inert, so that no bag-specific substances could contaminate the sample.

Afterward, the sample was put into a heating cabinet at 37°C imitating physiological conditions and the mass spectrometry was started using PTR-MS.

The Proton Transfer Reaction-Mass Spectrometry.

The analysis of samples was performed using PTR-MS. The samples were heated to 37°C. Subsequently, the gas in the sample bags was siphoned off by a heated Teflon tube (37°C). This technique uses H_3O^+ as a chemical ionization reagent to measure VOC in the parts per billion by volume (ppbv) to parts per trillion by volume (pptv) range. Protonated water, H_3O^+ , reacts with neutral molecules (M) according to $\text{H}_3\text{O}^+ + \text{M} \rightarrow \text{MH}^+ + \text{H}_2\text{O}$. This reaction only occurs if these neutral molecules have larger proton affinities than H_2O . Almost all VOCs have larger affinities, and therefore proton transfer occurs on every collision with rate constants k , having typical values of $1.5 \times 10^{-9} \text{ cm}^3 \text{ s}^{-1} < k < 4 \times 10^{-9} \text{ cm}^3 \text{ s}^{-1}$. The concentration of the protonated molecules (MH^+) is measured in the ion detection system. There is a linear relationship between the recorded MH^+ signal and the concentration of M in the original trace gas, so that the latter can be determined. The formula includes the normalized count rate of ions and of the primary ion H_3O^+ , the temperature in the drift tube, the pressure in the drift tube, the mass dependent transmission efficiency, the rate constant, and the reaction time.

Data. The PTR-MS differentiates in an online setting, the various VOC using their molecular weight. The measurable range is between 21 and 230 Da. This leads to 209 different values that are measurable in the following referred to as mass and the Daltons (eg, all substances with 69 Da are summed up with mass 69). The VOC are measured in the ppbv to pptv.

Each Teflon bag was measured at least 5 times. Therefore, the results for a patient were the mean value of a minimum of 15 measuring cycles.

Statistics. Statistical analysis was performed using a software package (SPSS, v.11.0, Chicago, IL). The mass concentrations are expressed as mean \pm SD in ppbv for each subgroup. Statistical significance was assumed at $p < .05$ and p values $< .01$ were considered highly significant.

The 4 subgroups were statistically analyzed with the Mann-Whitney U -test. Comparing the 4

study categories no statistical difference could be shown including all 4 test groups.

Therefore a Mann–Whitney *U*-test between the 2 most differing groups—the healthy control group and the carcinoma group—was performed and showed 42 masses with statistically significant *p* values.

The further statistical work-up was done comparing the different test groups using these 42 masses.

RESULTS

Initially a normal profile of VOC in the exhaled air of the healthy control group was created. This profile was compared with the 3 other study groups.

Two hundred nine different masses were measured; 188 of these were evaluated. Several masses (substances) are produced by the apparatus itself and so the latter cannot be used for statistical analysis and for the creation of the profile. To a large extent, the evaluated masses represent hydrocarbons, in particular alkanes, alkenes, alcohols, ketones, organic acids, etc. The mean concentration is given in ppbv.

The first step in analyzing the results was to calculate the mean value of each mass from the 3 different measurements.

As mentioned in the statistics paragraph, no statistically significant difference between the 4 groups could be shown. We compared the carcinoma group and the healthy control group using the Mann–Whitney *U*-test. This left us with 42 molecular masses showing a significant difference (*p* value >.05) between the 2 groups for further investigation. The masses are listed in Table 1. With these 42 values, a further descriptive statistical processing (Tables 2 and 3) was performed, considering mean value, minimal and maximal value, and standard deviation.

Thirty of the 42 masses showed a behavior similar to the 3 carcinoma-free study groups—control group, high-risk group, and posttherapy group—compared with a clearly higher or lower figure of the carcinoma group. Twelve masses showed an unexpected behavior. The exact distribution is listed in Table 4.

DISCUSSION

Several attempts analyzing the human breath for VOC have been described. So far, several pathologic alterations have been postulated in relation

Table 1. Different *p* values comparing the different groups.

Masses	Comparison of the different groups			
	A and C	B and C	D and C	A and B
29	.026	.67	.067	.883
30	.019	.61	.000	.118
31	.022	.34	.031	.883
32	.025	.50	.015	.574
37	.014	.32	.208	.807
38	.022	.32	.223	.883
39	.039	.53	.003	.807
42	.022	.05	.096	.000
44	.012	.36	.061	.942
46	.001	.14	.004	.980
56	.006	.12	.074	.406
57	.020	.67	.489	.608
64	.015	.14	.005	.732
65	.041	.58	.393	.845
68	.004	.14	.684	.009
69	.000	.60	.133	.136
70	.001	.00	.208	.171
72	.003	.012	.028	.448
74	.001	.019	.000	.406
86	.014	.030	.626	.660
87	.007	.016	.007	.696
88	.004	.009	.042	.807
89	.006	.015	.023	.769
90	.002	.017	.025	.788
94	.018	.013	.001	.448
100	.012	.089	.067	.353
102	.013	.044	.035	.678
116	.011	.004	.018	.696
117	.045	.032	.096	.942
128	.006	.007	.167	.883
136	.043	.004	.309	.261
143	.039	.111	.903	.448
153	.028	.937	.807	.018
157	.024	.089	.776	.293
163	.039	.044	.016	.854
178	.10	.453	.025	.055
187	.16	.664	.254	.026
193	.15	.303	.034	.264
198	.35	.438	.256	.261
209	.29	.226	.020	.398
223	.34	.116	.146	.818
230	.16	.559	.155	.033

Abbreviations: A, control group; B, high-risk group; C, carcinoma group; D, posttherapy group.

The *p* values <.05 were statistically significant.

to different diseases, like lung cancer¹ and schizophrenia.³ All these studies used a gas chromatography to analyze the different VOC.

In this study, we applied the PTR-MS for the first time to analyze the different VOC in the breath of tumor patients.

The first step evaluating the collected data was an internal quality control. This was provided by mass number 42, which stands for acetonitrile and has been shown to be directly correlated with

Table 2. Descriptive statistics: mean values, minimum/maximum values, and the standard deviation for the control group and the carcinoma group.

Masses	Control group			Carcinoma group		
	Mean value	Min./Max.	SD	Mean value	Min./Max.	SD
29	2.5342	0.9/4.43	0.97337	1.8726	0.99/3.71	0.87528
30	63.1155	42.75/95.23	14.45525	49.6511	15.6/95.82	20.71582
31	3.8734	1.28/8.55	1.92888	2.6749	1.12/6.44	1.58527
32	566.5381	337/1045	167.41916	460.8192	225/860	196.88
37	2636.2432	601/7849	2443.201	4811.2159	720/10916	3123.15
38	3.7883	0.56/11.85	3.60708	6.6319	1.04/13.86	4.14094
39	26.2118	7.01/146.9	27.81064	26.6906	11.43/44.72	7.85561
42	11.4018	2.64/5833	13.21793	31.3032	2.81/98.97	28.56943
44	11.9063	1.8/33.88	7.50278	7.5980	2.56/20.96	5.29358
46	34.7171	11.66/78.31	16.38132	21.2863	4.39/81.90	17.41283
56	0.4795	0.29/0.83	0.15056	0.3703	0.18/1.03	0.18662
57	18.6297	6.61/45.35	9.90951	12.5415	2.9/31.91	6.75590
64	0.2044	0.10/0.33	0.06746	0.1617	0.06/0.42	0.08356
65	0.4407	0.13/1.41	0.40280	0.6038	0.15/1.30	0.34414
68	0.4019	0.21/1.02	0.18866	0.5371	0.27/0.93	0.17739
69	48.2283	25.54/114.3	2.8783	78.7666	35.56/157.2	31.42997
70	2.8661	1.71/6.37	1.13701	4.3734	1.92/8.5	1.69452
72	0.7042	0.21/1.3	0.33833	0.4005	0.11/1.02	0.22565
74	4.4223	1.32/8.39	1.90810	2.6045	0.37/9.42	2.09584
86	0.4020	0.10/1.00	0.22174	0.2486	0.06/0.53	0.13678
87	6.4501	1.96/14.79	2.90176	4.1951	1.01/11.21	2.77198
88	1213.6171	0.51/3637.3	883.90121	612.1406	0.13/3683.8	872.3569
89	58.7564	1.57/182.15	41.31359	30.6849	0.86/159.43	38.0922
90	3.8674	1.06/11.37	2.31169	2.2396	0.14/9.81	2.22537
94	4.2926	0.32/10.53	2.68536	2.6246	0.17/10.94	2.58035
100	0.3226	0.12/0.53	0.10498	0.2451	0.08/0.55	0.10954
102	0.7082	0.22/1.15	0.29894	0.4708	0.11/1.1	0.32829
116	0.1460	0.03/0.32	0.07628	0.0992	0.04/0.16	0.03809
117	0.7687	0.13/1.50	0.36006	0.5775	0.08/1.93	0.41134
128	0.4026	0.02/1.99	0.40157	0.1867	0.04/0.59	0.16185
136	0.4836	0.10/0.93	0.25311	0.3236	0.02/0.78	0.24486
143	0.4430	0.05/1.62	0.45034	0.5996	0.12/1.8	0.44715
153	0.1069	0.0/0.3	0.07997	0.3902	0.01/2.57	0.58378
157	0.2042	0.0/0.91	0.2798	0.3686	0.03/1.05	0.30717
163	0.3004	0.0/0.80	0.21298	0.1837	0.03/0.74	0.15218
178	0.0044	0.0/0.03	0.00979	0.0081	0.0/0.04	0.01
187	0.0106	0.0/0.06	0.01976	0.0175	0.0/0.06	0.01804
193	0.0212	0.0/0.16	0.04245	0.0256	0.0/0.16	0.03512
198	0.0012	0.0/0.01	0.00290	0.003	0.0/0.01	0.00394
209	0.0027	0.0/0.03	0.00651	0.0063	0.0/0.02	0.00728
223	0.0180	0.0/0.08	0.03059	0.0328	0.0/0.09	0.03410
230	0.00	0.0/0.0	0.00000	0.0022	0.0/0.01	0.00349

the total number of smoked cigarettes.⁴ Our measurements showed a very low level of acetonitrile in the control group (mean value of 11.4 ppbv) and a low value in the posttherapy group (mean value of 16.9), whereas the high-risk group (mean value of 43.9 ppbv) had the highest, followed by the carcinoma group (mean value of 31.3). See Figure 1. These findings correlated perfectly with the patients' smoking behavior, at the measurement time all candidates of the high risk group and the carcinoma group being heavy smokers. The lower mean value of the carcinoma

groups may be explained by the fact that all tumor patients tried to reduce their smoking habits in the days after the diagnosis was established, a fact which underlines the correctness of the analysis.

The working hypothesis was that due to the altered metabolism⁶ in tumor patients, a change in the VOC profile of the breath should be detectable. We expected some masses to be significantly higher in the carcinoma group than in the control groups. This behavior could be proven for the masses 37, 38, 39, 65, 69, and 70.

Table 3. Descriptive statistics: mean values, minimum/maximum values, and the standard deviation for the high-risk group and the posttherapy group.

Masses	High risk group			Posttherapy group		
	Mean value	Min./Max.	SD	Mean value	Min./Max.	SD
29	2.4876	0.92/4.51	0.98748	18.0329	1.32/161.46	50.39841
30	55.1620	18.23/82.91	17.05561	89.7839	53.47/105.5	21.239
31	4.1841	0.84/11.38	2.63480	4.8726	2.52/16.87	4.26309
32	513.4526	220/877	168.1548	727.92	163.7/1021	293.2
37	2688.4355	735/7849.7	2498.813	2427.6125	1056/8031	2122.54
38	3.9027	1.06/10.70	3.63640	3.5766	1.5/12.32	3.30637
39	21.5004	8.52/35.92	8.44929	17.7399	10.53/36.87	8.41138
42	43.9079	5.92/100.05	26.96434	16.936	2.47/87.19	26.73531
44	11.0579	1.74/19.95	5.81931	10.2342	3.61/16.08	4.35411
46	36.8627	4.54/100.09	24.12719	39.21	14.31/57.77	14.406
56	0.9709	0.18/8.94	1.94343	0.4533	0.28/0.8	0.1574
57	16.0926	4.76/32.53	6.72852	13.6491	10.17/17.99	2.80319
64	0.2106	0.08/0.34	0.06997	0.2894	0.12/0.75	0.18288
65	0.6803	0.04/4.99	1.10101	9.5371	0.18/91.07	28.65058
68	0.8402	0.11/6.23	1.32506	0.5640	0.29/0.8	0.18636
69	61.4052	23.7/132.1	29.69963	63.1086	28.25/132.2	33.8
70	3.6419	1.51/8.04	1.72329	3.6836	1.61/7.58	1.95418
72	0.6108	0.17/1.05	0.25998	0.6017	0.36/1.11	0.23252
74	3.8724	0.46/8.07	1.90525	9.2366	3.95/26.85	6.62789
86	0.3608	0.07/0.79	0.17085	0.2573	0.18/0.47	0.09036
87	7.1490	1.02/16.12	4.21418	7.3552	3.52/11.85	2.63883
88	1468.85	0.22/5838	1418.57	980.5264	263.7/2295	582.57
89	67.2725	1.13/173.91	52.9358	55.1926	25.99/116	29.50812
90	4.2285	0.33/9.91	2.93804	3.4406	1.78/7.21	1.74265
94	5.0944	0.28/14.83	3.48191	6.35	1.48/10.32	2.57072
100	0.3074	0.11/0.64	0.12623	0.3123	0.2/0.47	0.08426
102	0.6835	0.12/1.17	0.29177	0.6906	0.45/1.04	0.18035
116	0.1458	0.05/0.25	0.05264	0.2488	0.08/1.33	0.38121
117	0.8767	0.26/2.65	0.58022	0.7038	0.41/1.11	0.26173
128	0.3593	0.07/0.87	0.25379	0.2322	0.1/0.55	0.13231
136	0.5906	0.06/1.33	0.30554	0.4186	0.24/0.86	0.18499
143	0.3974	0.11/0.9	0.23742	0.4823	0.28/0.63	0.10642
153	0.237	0.04/0.8	0.22191	0.1765	0.1/0.37	0.07946
157	0.2077	0.0/0.94	0.21941	0.2843	0.12/0.44	0.08616
163	0.2879	0.0/0.83	0.21104	0.3144	0.15/0.7	0.17642
178	0.0103	0.0/0.09	0.02174	0.0127	0.01/0.02	0.00455
187	0.0172	0.0/0.06	0.02083	0.023	0.0/0.07	0.01774
193	0.0243	0.0/0.13	0.03536	0.0362	0.01/0.09	0.0227
198	0.0026	0.0/0.01	0.00444	0.005	0.0/0.02	0.00532
209	0.0061	0.0/0.06	0.01383	0.0141	0.0/0.03	0.00945
223	0.0242	0.0/0.21	0.05112	0.0531	0.0/0.11	0.03543
230	0.0013	0.0/0.1	0.00251	0.0037	0.0/0.1	0.00208

Many of the different molecular masses, which are screened, are not yet fully understood. Therefore, it is very interesting that especially the mass 69 is found in higher concentrations in the carcinoma group (see Figure 2). Mass 69 also stands for isoprene. Isoprene is a frequently found VOC in the human breath.⁷ The origin of this substance stands in a close relationship to the cholesterol pathways.⁸ Furthermore, this VOC has been shown to be of bacterial origin.⁹ An interesting aspect of isoprene is its relation to oxidative stress.¹⁰ The last 2 facts could somehow

help to explain the significantly higher levels of isoprene in the breath of the carcinoma group, considering possible bacterial superinfection and activation of the immune system because of the neoplasm. This hypothesis still needs further investigation.

Another interesting finding was that certain masses showed a lower level of VOC in the carcinoma group, whereas all 3 carcinoma-free groups had a clearly higher count of ppbv. The following masses showed this described behavior: 29, 30, 31, 32, 44, 46, 56, 57, 64, 72, 74, 86, 87, 88, 89, 90, 94,

Table 4. The relationship between the different study groups according to the different masses.

Expected pattern			Unexpected pattern		
Carcinoma group higher	Carcinoma group lower		High-risk group and carcinoma group high	Posttherapy group high	High-risk group and posttherapy group high
37	29	30	42	143	68
38	31	32		157	153
39	44	46		193	178
65	56	57		198	187
69	64	72		209	
70	74	86		229	
	87	88		230	
	89	90			
	94	100			
	102	116			
	117	128			
	136	163			

100, 102, 116, 117, 128, 136, and 163. For us this fact is explainable with the typically cancer-associated catabolic metabolism and the cancer-associated cachexia.⁵ So far, no further data to explain these findings in detail is available.

Nevertheless, some of these masses, which should have a certain level of ppbv in order to guarantee a minimum amount of accuracy, could be perfectly usable for a screening test. From our point of view, the following masses measuring above 1.0 ppbv, like 29, 30, 31, 32, 44, 46, 56, 57, 64, 72, 74, 86, 87, 88, 89, 90, and 94, would be suitable for use in a screening procedure.

Twelve of the 42 significant different masses, which showed an unexplainable pattern, are: 42,

68, 143, 153, 157, 178, 187, 193, 198, 209, 229, and 230. The mass 42, standing for acetonitrile, is the only 1 that is directly explainable by the subjects' lifestyle. The others are measured in a very low concentration, under 1.0 ppbv, suggesting a questionable measuring accuracy. Although the patients fasted for about 12 hours prior to the measurement, it is still possible that nutrients consumed the day before influenced the test result.¹¹

The number of open questions emphasizes the need for further research, until a usable screening method for ENT tumors is developed. Nevertheless, an oncologic diagnostic tool consisting of simple breath analysis is very tempting.

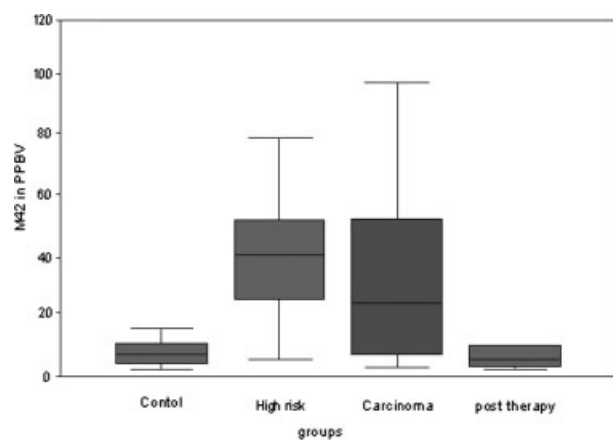


FIGURE 1. A box plot of mass 42, which stands for acetonitrile is shown here. Acetonitrile was increased in the high-risk group and the carcinoma group. All subjects in both groups were heavy smokers.

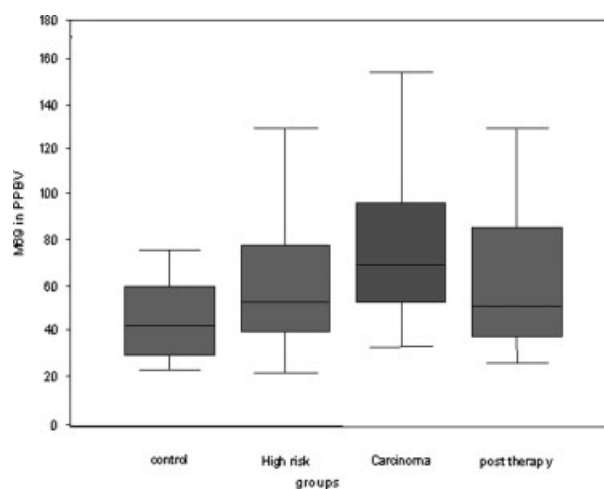


FIGURE 2. A box plot for mass 69, which stands for isoprene, is shown here.

CONCLUSION

A screening method for HNSCC using VOC seems to be possible. Still, further investigations are necessary to establish this procedure for routine usage.

REFERENCES

1. Phillips M, Gleeson K, Hughes JM, et al. Volatile organic compounds in breath as markers of lung cancer: a cross-sectional study. *Lancet* 1999;353:1930–1933.
2. Phillips M, Cataneo RN, Ditkoff BA, et al. Volatile markers of breast cancer in the breath. *Breast J* 2003;9:184–191.
3. Phillips M, Erickson GA, Sabas M, Smith JP, Greenberg J. Volatile organic compounds in the breath of patients with schizophrenia. *J Clin Pathol* 1995;48:466–469.
4. Lirk P, Bodrogi F, Deibl M, et al. Quantification of recent smoking behaviour using proton transfer reaction-mass spectrometry (PTR-MS). *Wien Klin Wochenschr* 2004;116:21–25.
5. Esper DH, Harb WA. The cancer cachexia syndrome: a review of metabolic and clinical manifestations. *Nutr Clin Pract* 2005;20:369–376.
6. Dang CV, Semenza GL. Oncogenic alterations of metabolism. *Trends Biochem Sci* 1999;24:68–72.
7. Rieder J, Lirk P, Ebenbichler C, et al. Analysis of volatile organic compounds: possible applications in metabolic disorders and cancer screening. *Wien Klin Wochenschr* 2001;113:181–185.
8. Karl T, Prazeller P, Mayr D, et al. Human breath isoprene and its relation to blood cholesterol levels: new measurements and modeling. *J Appl Physiol* 2001;91:762–770.
9. Kuzma J, Nemecek-Marshall M, Pollock WH, Fall R. Bacteria produce the volatile hydrocarbon isoprene. *Curr Microbiol* 1995;30:97–103.
10. Mendis S, Sobotka PA, Euler DE. Expired hydrocarbons in patients with acute myocardial infarction. *Free Radical Res* 1995;23:117–122.
11. Rosen RT, Hiserodt RD, Fukuda EK, et al. The determination of metabolites of garlic preparations in breath and human plasma. *Biofactors* 2000;13:241–249.