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Applications of breath gas analysis in medicine☆

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Abstract

Volatile organic compounds (VOCs) in exhaled breath gas provide valuable information about the subjects' physiological and pathophysiological condition. Proton-transfer-reaction mass spectrometry (PTR-MS) allows rapid and online measurements of these substances. We present results of three studies illustrating the potential of breath gas analysis by PTR-MS in various contexts: long-time online monitoring of VOCs in sleeping subjects suggests that VOC profiles are related to sleep stages. Analysis of VOC concentrations in the breath of carbohydrate malabsorbers emphasizes the role played by bacteria in the gut. Finally, we demonstrate the large intra- and intersubject concentration variability of VOCs by considering one particular mass.

Keywords: Breath test; Proton-transfer-reaction mass spectrometry (PTR-MS); Volatile organic compound (VOC); Sleep

1. Introduction

The importance of analyzing volatile organic compounds (VOCs) in human breath gas for biomedical research has earlier been recognized [1–3]. In the past decades, numerous studies have been performed in order to establish the physiological and pathophysiological meaning of VOC emission in human breath gas [4–8]. Results obtained in these studies indicate that the analysis of VOCs in breath gas may become a promising non-invasive tool for medical diagnosis and for monitoring the success of therapy.

VOCs are present in very low concentration in human breath gas, at the level of parts per billion by volume (ppbv) and even lower. An investigation of the breath of 50 individuals revealed about 3400 VOCs with an average of 204 substances per person [9]. The origin and physiological function of most of them, however, are still not known. Some VOCs in breath gas originate from the subject's previous exposure to precursors. In this case, the precursors are first taken up via inhalation, ingestion or skin contact, then possibly metabolized, and finally excreted by expiration. Other VOCs are genuine metabolites of the organism itself or of its intestinal bacteria. In that case, they are conveyed to the lungs by the blood circulation and excreted by expiration. VOC concentrations vary depending on food intake, state of physical condition, general health of the subject, and multiple environmental factors. This suggests that intra- and intersubject variability in VOC emissions is substantial, as confirmed in [9].

Breath gas analysis is difficult to perform because of low VOC concentrations in breath air, high humidity of breath gas, lack of suitable sampling techniques, and absence of sen-

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sitive and capable measuring techniques. Meanwhile these difficulties have partly been overcome by improvements in sampling and analytical techniques. Preconcentrating breath gas by various means and subsequent analysis by means of gas chromatography combined with mass spectrometry (GC/MS) constitute a reliable and sensitive set of methods for VOC analysis [2,10–12]. Despite its high sensitivity, the GC/MS technique has considerable drawbacks: it is time-consuming, difficult to handle, and not suitable for online and multiple measurements.

Analyzing breath gas by proton-transfer-reaction mass spectrometry (PTR-MS) has significant advantages: gas mixtures may be readily analyzed without previous concentration and separation procedures; compounds occurring in high concentration like N_2, CO_2, O_2, H_2O do not interfere with measurement; the instrument has very high sensitivity (down to parts per trillion by volume, pptv); frequent and rapid measurements are possible. PTR-MS is, therefore, a promising technique for VOC analysis in breath gas particularly suited for online and multiple measurements. Note, however, that PTR-MS characterizes the substances solely according to their mass-to-charge ratio; chemical identification is thus, not possible and must be provided by other, more tedious techniques.

This paper presents the results of three studies investigating VOC emission patterns using the PTR-MS technique: (1) long-time, online monitoring of VOC profiles during sleep combined with polysomnography; (2) analysis of VOC patterns in patients suffering from carbohydrate malabsorption; (3) analysis of intra- and intersubject variability of one particular mass. The purpose of the first study was to investigate VOC patterns during sleep and to find correlations between sleep stages and VOC patterns. The second study aimed at establishing patterns of VOCs which are characteristic for malabsorption syndromes. The purpose of the third study was to investigate diurnal fluctuations of one mass suspected to be a biomarker for lung carcinoma.

2. Materials and methods

2.1. Subjects

All studies were approved by the local ethics committee, and informed consent of the patient or the patient's nearest relative and the members of the control groups was obtained.

2.1.1. Sleep monitoring

We have studied the sleep patterns of 20 healthy males individuals, aged 20–30 years, who rested in bed from approximately 11 p.m.–7 a.m. All subjects underwent medical checkups for general health and sleep disorders.

2.1.2. Carbohydrate malabsorption

Seventeen adult outpatients, aged 25–50 years, were examined for malabsorption syndromes. Subjects were included if any of the following gastrointestinal symptoms were present: stool irregularities, bloating, abdominal cramps, diarrhea, constipation or nausea, but otherwise healthy. Physical examination and routine laboratory assessment did not reveal abnormalities. None of the patients showed signs of inflammatory bowel disease, any other chronic disease or infectious diseases. The subjects were not taking any medication.

2.1.3. Intra- and intersubject variability of mass 108 u[1]

In order to investigate the natural concentration variability of mass 108 u in breath gas, we examined four different groups of subjects.

Group 1 included 17 lung carcinoma patients with radiologically and clinically proven diagnosis of lung carcinoma. A total of 33 samples were analyzed. Group 2 consisted of 41 healthy individuals (staff nurses, anesthetists), working in operating rooms, aged 21–57 years. Their breath was sampled at the end of their working day and the following morning. A total of 207 breath samples were analyzed. Group 3 consisted of 32 healthy individuals, not working in clinical environment. A total of 32 breath samples were obtained. Group 4 included 11 healthy individuals, aged 25–48 years, working at Innsbruck University Psychiatric Clinic. Their breath was sampled at regular intervals during day-time (at 8, 11, 14, 17, and 20 h). A total of 48 samples were obtained.

In reference groups 2–4, subjects suffering from lung disease, cancer, depressive and metabolic disorders were excluded.

2.2. Experimental

2.2.1. Measurement of the VOCs

Proton-transfer-reaction mass spectrometry, developed for online trace gas monitoring [13,14], combines the concept of chemical ionization introduced by Munson and Field in 1966 [15] with the swarm technique of the flow drift tube (FDT) invented by Ferguson and his colleagues in the early seventies [16]. The device is extensively described and discussed in the current volume; its detailed description is therefore, omitted here.

2.2.2. Breath sampling

For the online measurements in the sleep laboratory, breath gas was collected through a mask covering the nose and mouth, from which it was transferred to the PTR-MS device. The perfluoroalkoxy copolymer transfer line was heated to prevent water condensation that may cause memory effects.

For the studies of carbohydrate malabsorption and intra- and intersubject variability, we collected breath gas in 3-L

[1] Substances detected by PTR-MS are all protonated. Hence the mass of the detected ion equals the mass of the substance plus the mass of one proton, m_H (= 1 u). We refer to a particular substance by the mass of the protonated species. "u" is the recommended symbol for the unified atomic mass unit.

Tedlar® bags (made from polyvinylfluoride): the subjects, who first sat at rest for 5–10 min, inflated the bag with one to two breaths. A reference sample of ambient (inhaled) air was collected where the patient was sitting. To prevent condensation, the breath samples as well as the transfer line were heated to 42 °C before and during PTR-MS measurements. The breath samples from the malabsorption patients were collected at 30-min intervals, starting with carbohydrate administration.

2.2.3. H_2 breath test for carbohydrate malabsorbers

Hydrogen breath tests [17] were performed using a Bedfont gastrolizer (Bedfont Ltd., Kent, UK) and were based on electrochemical detection of hydrogen gas. After a 12-h overnight fast a baseline H_2 breath test was performed. An oral dose of 25 g fructose or 50 g lactose (the carbohydrate suspected not to be absorbed by the patient) was given in 250 ml tap water. H_2 exhalation was monitored at 30-min intervals for at least 2 h. All tests were started between 8:00 h and 8:30 h. Maximum H_2 exhalation after carbohydrate load was monitored and the differences to baseline levels (ΔH_2) were calculated. Individuals with ΔH_2 higher than 20 ppm (parts per million) after the fructose/lactose load were classified as malabsorbers.

2.2.4. Sleep scoring

The sleep laboratory is part of Innbruck University Psychiatric Clinic. Electrophysiological parameters were recorded with a Nihon Kohden electro-encephalograph 4317 F.

Sleep was classified in five stages according to the rules proposed by Rechtschaffen and Kales [18] based on recognizable brain activity (EEG), eye motion (electro-oculogram, EOG) and muscle activity (electro-myogram, EMG), as recorded by a polysomnograph. Light sleep corresponds to stages 1 and 2, deep sleep to stages 3 and 4, rapid eye movement (REM) sleep to stage 5. Additionally, we refer to the waking state (awake, but lying in bed) as stage 0.

2.2.5. Data analysis

The data were evaluated using MATLAB software (Version 6.5.0.180913a (R13), The Mathworks, Natick, Massachusetts (USA)) on a basic statistical level.

3. Results

3.1. Sleep monitoring

Fig. 1 shows the concentrations for masses 45 u (= m_1 + m_H) and 59 u (= m_2 + m_H) for one particular night: m_1 corresponds (primarily) to acetaldehyde (carbon dioxide with the same mass is not detected by PTR-MS), m_2 to acetone. Acetaldehyde is an oxidation product of ethanol. Its concentration decreases during sleep. This variation in concentration is typical for many VOCs. Acetone, a degradation product of acetyl-CoA when fat breakdown predominates, exhibits

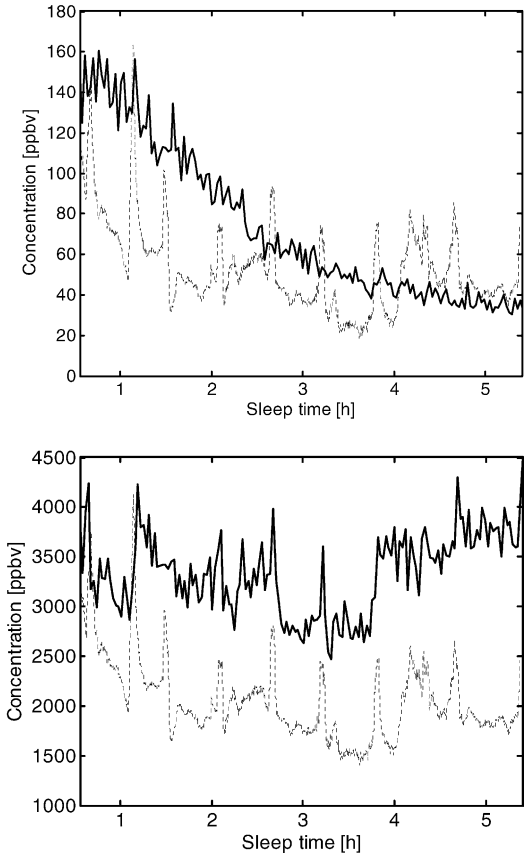

Fig. 1. Upper panel: concentration of mass 45 u corresponding to acetaldehyde (solid) and heart rate (dashed, to an arbitrary scale) as a function of time during one particular night. Lower panel: same data for mass 59 u corresponding to acetone.

a more complicated pattern with an minimum plateau during the middle of the sleep period; during the second half its concentration increases, possibly indicating hunger.

Mass 69 u, corresponding (primarily) to isoprene, is related to the biosynthesis of cholesterol and is one of the most abundant substances in breath gas. Its time course in the exhaled air shows a variable pattern, depending on the cardiac output and therefore, correlates with heart rate (Fig. 2) and probably also with breath volume. This behavior is also observed for some other masses, but is most pronounced at mass 69 u.

Oxidation of ethanol (observed mass m = 47 u) to acetaldehyde (observed mass m = 45 u) by the enzyme alcohol dehydrogenase is readily monitored by PTR-MS. Fig. 3 shows an increase in the ratio of acetaldehyde to ethanol concentrations during the night for various subjects.

Table 1 shows, for one particular night in one subject, the number of transitions between the sleep stages and the corresponding changes in median values of isoprene concentration (the median value for each sleep stage was obtained by taking into account all sleep stages of this type during the particular night): for the particular night shown, the transitions from stage 0 to 1 are associated with an increase in median iso-

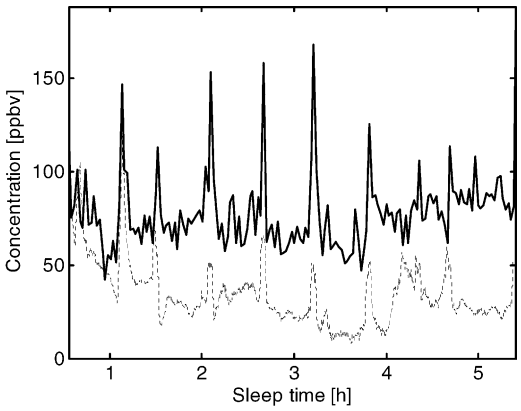

Fig. 2. Concentration of mass 69 u corresponding to isoprene as a function of time for the same night as in Fig 1. For details, see Fig. 1. Note the characteristic variation depending on heart rate. For further details, see text.

prene concentrations by a factor of about 1.21, whereas the transitions from stage 5 to 0 are associated with a decrease by a factor of 0.61.

3.2. Carbohydrate malabsorption

Fig. 4 shows the time variation in mass 63 u concentration after carbohydrate administration, obtained for 17 patients. Malabsorbers were detected by hydrogen breath test (results not shown); the corresponding results are depicted in bold symbols/lines. Concentration of mass 63 u in the breath gas of the malabsorbers with non-gastrointestinal symptoms (such as vertigo, headache, sleepiness, hypotension) increases 90 min after carbohydrate administration, whereas this trend is not seen in the breath of the other subjects.

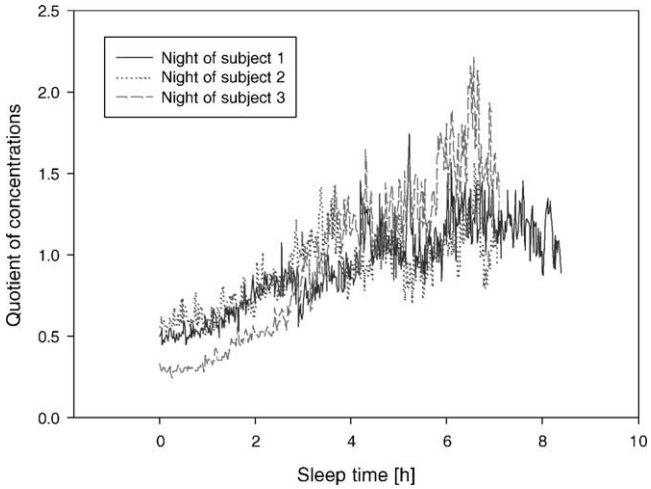

Fig. 3. Ratio of acetaldehyde (mass 45 u) to ethanol (mass 47 u) concentrations during three nights. The ratio increased with time as expected when ethanol metabolizes to acetaldehyde.

Table 1
Number of transitions between sleep stages and ratio of the corresponding median isoprene concentrations

From	To					
	0	1	2	3	4	5
0	–	16/1.21	1/1.00	–	–	1/0.95
1	4/1.10	–	12/1.10	–	–	–
2	10/0.78	–	–	23/0.97	1/0.98	1/1.03
3	1/0.70	–	21/1.01	–	11/0.99	1/1.20
4	–	–	1/0.87	11/0.99	–	–
5	3/0.61	–	–	–	–	–

For instance, there were 16 transitions from stage 0 to 1; they were accompanied by an increase in the median isoprene concentration by a factor of 1.21. "–" indicates that no transitions occurred for the corresponding pair of sleep stages.

3.3. Intra- and intersubject variability of mass 108 u

Fig. 5 shows the breath gas concentration of mass 108 u in subjects belonging to the four groups specified above. The mean value found for these groups does not show any statistically significant difference. In particular, lung carcinoma patients do not exhibit higher concentrations of mass 108 u than the control groups. Scattering is lowest for the control group including subjects not working in clinical environment; in all other groups there are many outliers.

For subjects in the hospital control group 2, five breath samples were taken per day on two consecutive days. The results of the first day, altogether 49 samples, are depicted in Fig. 6 (the records of the second day are incomplete and therefore, omitted). The concentration of mass 108 u exhibits considerable diurnal variation in some subjects: it shows abnormally high values at t = 11 and 17 h for some subjects, while it is lower than 2 ppbv at all other times.

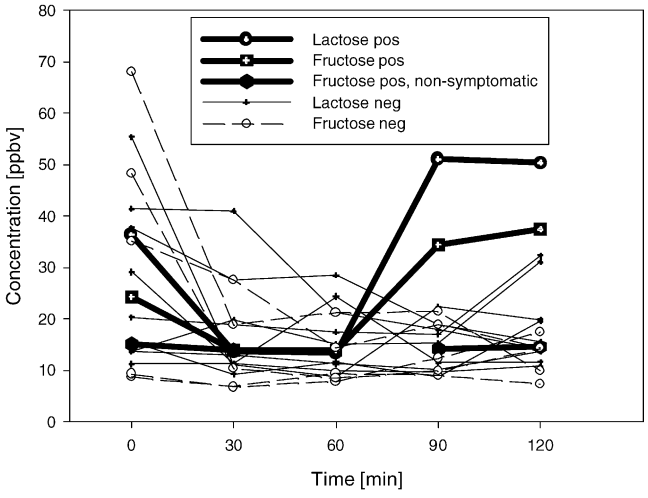

Fig. 4. Concentration of mass 63 u, probably corresponding to a sulfur-containing substance, as a function of time for 17 patients. Each curve designates a single patient. Malabsorbers are indicated by bold symbols/lines. Time t = 0 min designates the time of carbohydrate administration.

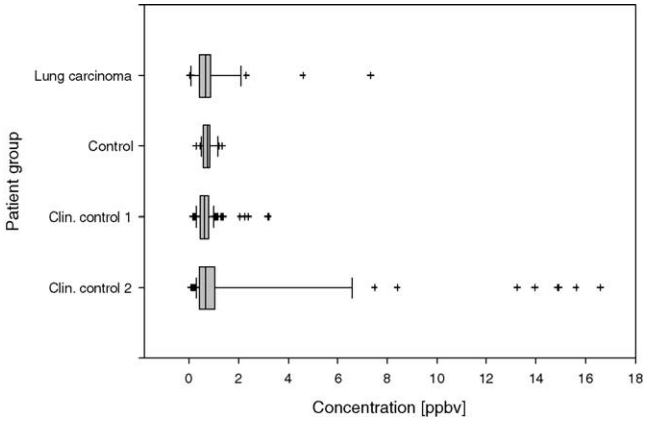

Fig. 5. Concentrations of mass 108 u (including *o*-toluidine) in the breath of 17 lung carcinoma patients (lung carcinoma), 32 healthy individuals working outside the clinical environment (control), 11 and 41 healthy individuals working inside the clinical environment (Clin. contr. 1 and Clin. contr. 2). The boxes have lines at the lower quartile, median, and upper quartile values. The whiskers show the 10 and 90 percentiles. Outliers (+) are values beyond the end of the whiskers. For details, refer to the text.

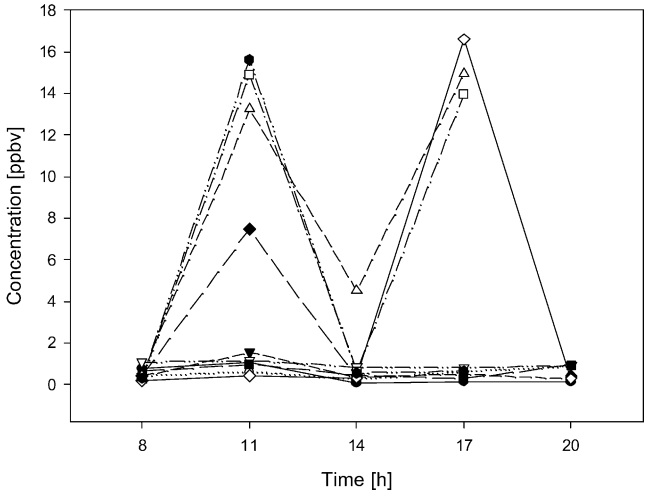

Fig. 6. Concentration of mass 108 u in the breath of 11 healthy individuals (corresponding to clin. contr. 1 of Fig. 5 as a function of sampling time during 1 day. Each line corresponds to one subject. Note that some subjects did not deliver five samples. For details, see text.

4. Discussion

4.1. Sleep monitoring

The monitoring of metabolic processes over several hours may give an insight into physiological and pathophysiological processes. Sleep provides ideal conditions for such long-duration online monitoring (typically up to 8 h), which is difficult to perform on awake individuals due to interference with food intake and other daily activities.

We observe breath VOC concentrations varying with time. Some concentrations increase with time, some decrease, others have stepwise variation, still others strongly depend on heart-rate and breath volume. This is particularly striking in the case of mass 69 u, representing primarily isoprene. Relevant questions in this context are: how does the concentration of isoprene *in blood* relate to its concentration in breath gas? To what extent does its blood concentration also depend on heart rate and breath volume? Further work, both experimental and modelling, is required to clarify the unusual behavior of isoprene (cf. [19]).

Investigations by Cailleux [20] and DeMaster [21] have shown that isoprene increases during sleep and decreases after awakening. Our findings indicate a more subtle behavior: apart from the increase due to higher heart rate, isoprene may increase during sleep, but not to the extent proposed by Cailleux and DeMaster. The pronounced effects observed by these groups are most likely a consequence of their non-sleep-preserving experimental design: as the probands were repeatedly awakened to give each single-breath sample, the higher isoprene concentrations observed in the probands' breath gas were probably an effect of their heart rate that rose due to the awakening stress.

Our results on ethanol oxidation (see Fig. 3) show that biochemical processes may be monitored online by PTR-MS. The sensitivity of the method is such that even residual ethanol concentrations can be traced.

The biochemical correlates or triggers of the sleep stages are still unknown, mainly due to the lack of online monitoring methods. Our hypothesis is that the underlying biochemical pathways are reflected by VOC profiles as measured by PTR-MS. In general, not only a few, but an intertwined network of physiological reactions is involved, making analysis much more difficult than with ethanol degradation. In a first step, we investigated the changes occurring in selected VOCs at sleep stage transitions. These non-cross-validated preliminary results, the first of their kind, encourage further research in this direction.

4.2. Carbohydrate malabsorption

Carbohydrate malabsorption is a condition in which patients are unable to absorb or digest certain carbohydrates due to the lack of one or more intestinal enzymes [22] or transport systems [23]. Therefore, the carbohydrates remain in the intestinal lumen and, for osmotic reasons, lead to fluid retention, causing diarrhea and abdominal distention. Bacterial sugar fermentation in the gut leads to gaseous and acidic stools. Well-documented syndromes are lactose maldigestion [17] (lactase deficiency: lactose cannot be split into galactose and glucose) and fructose malabsorption [23] (a defect of the fructose-related GLUT-5 transport system); about 80% of the lactose maldigesters also suffer from fructose malabsorption. Bacteria in the gut metabolize the carbohydrates to low-molecular species, such as carbon dioxide, short-chained fatty acids, esters, alcohols and hydrogen, which are exhaled in the breath. Routine diagnosis is carried out by measuring the hydrogen content in the breath after oral administration of the sugar suspected not to be absorbed. Hydrogen con-

centration higher than 20 ppm above baseline is indicative of malabsorption/maldigestion. This level is achieved after a latency of about 60–90 min. It should be noted that not all patients who have the mentioned enzyme-deficiency exhibit gastrointestinal and systemic symptoms: occurrence of the symptoms may depend on the species and quantity of bacteria in the gut.

It is well known that some patients suffering from carbohydrate malabsorption syndrome have sulfur reducing bacteria in their gut [24]. Therefore, we looked at sulfur containing VOCs in the breath gas of such subjects. Ethanethiol or dimethylsulfide, both having mass 63 u, are promising candidates for such VOCs. Mass 63 u has fairly low concentrations in healthy individuals, about 10 ppbv (not shown).

In malabsorbers (with one exception), the concentration of mass 63 u is indeed elevated 90 min after carbohydrate administration, while non-malabsorbers have lower concentrations, see Fig. 4. The malabsorber whose breath does not contain an elevated concentration of mass 63 u after 90 min, was also the only one who did not show any pronounced change in symptoms after carbohydrate administration.

The data shown are preliminary results based on a small group of subjects. Nevertheless, they suggest that one may distinguish between symptomatic and symptom-free patients suffering from malabsorption/maldigestion through breath gas analysis.

4.3. Intra- and intersubject variability of mass 108 u

Preti et al. found *o*-toluidine (*o*-methyl-aniline, mass 107 u, observed by PTR-MS at mass 108 u) in significantly greater concentrations in the breath gas of patients with lung carcinoma than in that of an age-matched control group. It was therefore suspected to be a marker for bronchial carcinoma [25]. Earlier investigations by our group [8] suggested that mass 108 u is significantly elevated in carcinoma patients as compared to healthy individuals. This result appeared to corroborate the findings of Preti et al.

Since then, we have conducted several studies in an enlarged collective of diseased and healthy individuals working in- and outside a clinical environment. Fig. 5 shows no difference in the concentration of mass 108 u between healthy subjects and carcinoma patients. This disproves our previous conclusion inferred from comparison with merely one control group. Thus mass 108 u is inappropriate as a biomarker for lung carcinoma. Since PTR-MS detects merely the sum of all present species of mass 108 u, the observed mass-108-u signal does not necessarily originate exclusively from *o*-toluidine. It is necessary to identify the substances contributing to mass 108 u by means of GC/MS in order to clarify this matter.

Additionally, the substantial diurnal variation in mass 108, as shown in Fig. 6, suggests that the exact sampling time may be crucial for consistent interpretation of the data.

5. Conclusions

Our conclusions are:

1. PTR-MS is a powerful technique for online VOC monitoring. It affords a new opportunity for non-invasive online observation of biochemical reactions in the body, especially during sleep. This gives an insight into metabolic processes not previously accessible (Figs. 1–3).
2. VOC profiles are related to sleep stages (Table 1).
3. Hemodynamics and pulmonary functions play a role in interpreting the VOC concentrations, particularly for the derivation of the substance concentrations in blood (Fig. 2).
4. Bacteria in the gut produce VOCs. This must not be forgotten when interpreting the data (Fig. 4).
5. Mass 108 u is not a reliable biomarker for lung carcinoma (Figs. 5 and 6).
6. Interpretation of VOC concentration profiles for subjects in a clinical environment requires special care (Fig. 6).

Further investigations of the following topics are in progress or planned:

1. VOC profile investigation of patients suffering from sleep disorders and probands under sleep withdrawal: this will give further insight into the biochemical mechanisms accompanying sleep.
2. Breath gas analysis in a large number of patients suffering from malabsorption syndromes: this will further elucidate the role of bacteria in the gut and may help distinguish between symptomatic and non-symptomatic malabsorbers.
3. Collection of a reliable set of VOC reference data from normal, healthy subjects, taking into account diurnal VOC variation and workplace exposure to typical chemicals (especially in the clinical environment): this will provide reliable comparative data.
4. Simulation and modeling of hemodynamics and pulmonary functions: this will provide clues for determining the VOC concentration in blood based on their concentration in breath gas.
5. Complementary breath gas analysis by GC/MS: this will chemically identify the substances, thus refining the results obtained by PTR-MS.
6. Comparative breath gas analysis in patients suffering from specific diseases: comparison with the reference set (point 3 above) will reveal potential biomarkers for disease diagnosis.

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