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Breath analysis for medical diagnosis and therapeutic monitoring

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The physical, biochemical and molecular biological methods of medical diagnostics have been developed very rapidly in recent decades. The main focus has been on blood and urine diagnostics. Diagnostics based on exhaled human breath or head-space of urine, on the other hand, is much less developed and not yet widely utilised in clinical practice. Nevertheless, it has long been known that certain diseases go along with a characteristic smell, which may be due to exhaled breath, sweat or other sources.

In modern medicine, diagnosis based on smells has been suppressed for many years as being subjective and unreliable. Due to new or improved analytical methodology, smell-based diagnostics is undergoing a revival. Having quantitative and very sensitive analytical methods at hand, it has become possible to develop smell-based medical diagnoses. A recent study using dogs trained to smell bladder or prostate cancer from urine of the respective patients gives another hint into this direction.

The analytical methodology today is based on:

- gas chromatography with mass-spectrometric detection (GC-MS);[1]
- mass spectrometric methods using chemical ionisation such as proton-transfer-reaction mass spectrometry (PTR-MS), selected ion flow tube mass spectrometry (SIFT-MS) or ion–molecule reactions (IMR). Among the pioneers of these techniques are Werner Lindinger (PTR-MS),[2] David Smith (SIFT-MS)[3] and Johannes Villinger (IMR);[4]
- laser spectrometric methods such as infrared cavity leak-out spectroscopy (CALOS)[5] or tunable diode laser absorption spectroscopy (TDLAS) in combination with quantum cascade lasers (QCLs);[6]
- or ion mobility spectroscopy.[7]

It is not only the analytical methodology that has been developed, but also the sampling procedures for exhaled breath or urine headspace are being increasingly investigated and refined. A particularly promising technology used for gas-chromatographic investigations is solid-phase microextraction (SPME) developed by Janusz Pawliszyn.[8]

Volatile compounds in breath are produced by metabolic processes at various organs and places in the body, by bacteria in the gut or both. Nitric oxide (NO) is a particularly interesting and instructive example. It was detected as endothelium-derived relaxing factor (EDRF) and identified later as NO. These discoveries led to the award of the Nobel Price in Physiology and Medicine to Murad, Furchtgott and Ignarro in 1998. Nitric oxide arises in virtually all organs of mammals, and is, in particular, produced in the airways and the paranasal sinuses. Actually, the concentration in the paranasal sinuses, where it acts as a bacteriostatic agent, is much higher than in the airways itself. Measurement of NO in the airways therefore needs a careful protocol, which has been suggested by the American Thoracic Society. Nitric oxide can be used, for example, for therapeutic monitoring of asthma patients, for the detection of primary ciliary dyskinesis (Kartagener's syndrome) or infections of the respiratory system. It is responsible for the hypoxic vasoconstriction of the respiratory system and plays an extensive role throughout the vascular system of the body. The concentration of NO may change quickly, as, for example, after a challenge with acetylcholine or substance P, or even when humming as compared with silent exhalations. Among the pioneers in the field are Lars Gustafsson, Jon Lundberg and Eddy Weitzberg at the Karolinska Institutet in Stockholm.

Another example of an interesting volatile compound is isoprene (2-methyl-1,3-butadiene), produced from dimethyl allyl pyrophosphate, an intermediate compound in the cholesterine synthesis pathway. Its concentration is related to the blood cholesterin and LDL levels and depends on heart rate, breathing rate and breathing volume. (LDL is low-density lipoproteins, often referred to as "bad" cholesterol). It could potentially be used for diagnosis and clinical monitoring of dyslipidemias. To do so, a careful understanding of the detailed effects of lung mechanics and hemodynamics and their influence on the concentration of isoprene in breath is necessary.

In Figure 1, the concentration of isoprene for a volunteer during a full night is shown, together with the smoothed pulse frequency (in arbitrary units). The

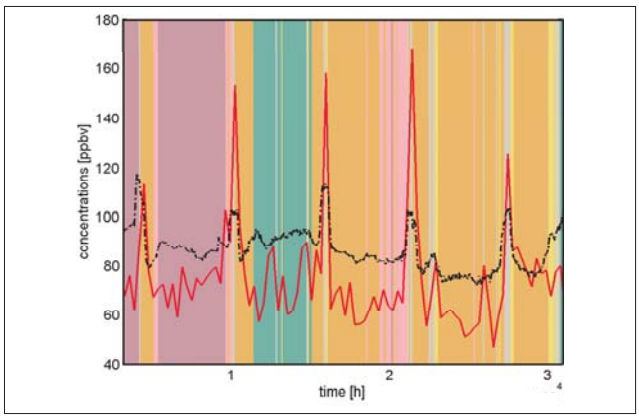

Figure 1. On-line measurement of isoprene with a protonated mass of 69 Da for one healthy volunteer during three hours of a night (red line). Measurements have been performed using PTR mass spectrometry. The x-axis gives time (h), the y-axis concentration in parts-per-billion (10^{-9}). For comparison, the smoothed pulse frequency is shown (black dotted line) in arbitrary units. Increasing heart rate leads to an increase in exhaled isoprene concentrations, whereas an increase in breathing frequency leads to a decrease in exhaled isoprene concentrations (not shown). The sleep stages are given in a colour code (awake: gray, light sleep stages: yellow and ochre, deep sleep stages: magenta, REM sleep: green).

coloured background of the figure represents the sleep stages, with rapid eye movement (REM) sleep coloured green. The correlation between pulse frequency and isoprene concentration is clearly visible. The breath gas measurements in this example have been performed using PTR mass spectrometry, not only recording the concentration of isoprene, but also of many other components related to the biochemical status of the sleeping body. It illustrates the possibility to sample breath continuously and analyse the samples in an on-line manner. This may be done after a challenge test, at the ergometer or in the sleep laboratory.

On-line measurements are very useful for understanding the influence of lung mechanics, hemodynamics and blood-gas kinetics on exhaled breath concentration patterns. They also provide the basis for getting additional information about the sources of a particular substance in the body, and to model the respective substance mass flows between different body compartments. Model calculations can, for example, be made for toxic substances in the environment such as, for example, methyl tertiary butyl ether (MTBE), which accumulates in fat compartments of the body. MTBE was introduced to replace lead in gasoline as an octane enhancer and comprises 1–5% of automotive fuel.

For a few volatile substances in breath, the biochemical origins are known or can at least be suspected. A recent example of an interesting substance is 3-heptanone in the breath of pediatric patients suffering from propionic acidaemia (PA), caused by a deficiency in propionyl-CoA carboxylase. For patients suffering from PA, no obvious quantitative parameter for "quantification" of

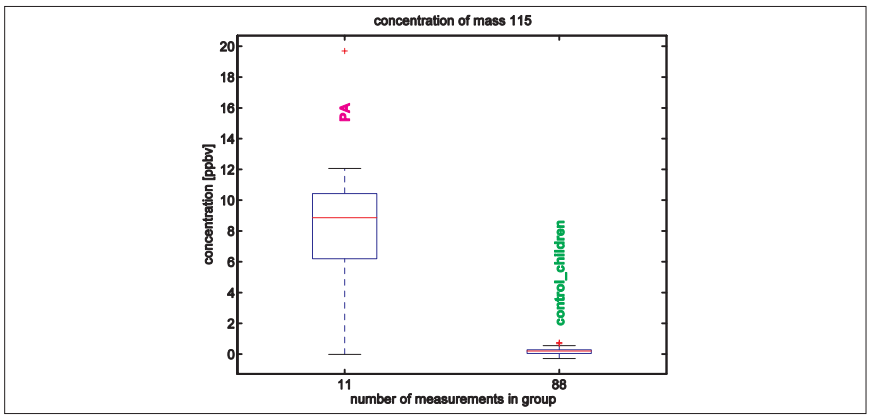

Figure 2. Concentration of 3-heptanone in parts-per-billion for pediatric patients suffering from propionic acidaemia as compared with healthy age-matched controls. The red lines are the respective median concentrations, the blue boxes refer to the lower and upper quartile values. The read "+" is an outlier (with an exceptionally high concentration). Measurements were carried out using PTR mass spectrometry with a GC-MS library identification (Wiley 7N library).

disease severity is known and one must rely on the clinical picture (ketoacidosis, seizures, vomiting, lethargy and hypotonia). Hence additional parameters like concentration of 3-heptanone in exhaled breath might in the future become useful as non-invasive and easily determinable parameters for disease severity and therapeutic control of appropriate diet. A hint in this direction comes from the fact, that one of our PA-patients with normal values of 3-heptanone presents a comparatively good clinical picture.

Only in rare cases is the distinction between a certain disease and normal healthy volunteers as pronounced as shown in Figure 2. In cancer screening, for example, one usually combines different substances' concentrations into one parameter which indicates if cancer should be suspected or not. Michael Phillips[9] has, for example, identified nine different alkanes (e.g. 7-methyl-tridecane) as being important for detection of lung cancer patients. The difference in concentration in exhaled and inhaled air of these substances is then combined by appropriate statistical algorithms into a risk parameter for lung cancer. The thresholds used for this risk parameter are chosen to result in a high corresponding specificity: persons classified as having no risk for cancer can then safely be sorted out and need not undergo further more invasive checks involving lung or heart biopsies.

Breath analysis is a very interesting, non-invasive method with high potential for medical diagnosis, therapeutic monitoring and physiological measurements. The fast development of analytical techniques will lead to reliable, miniaturised devices useful for the clinical practitioner in many medical fields in the next years. An overview of the field is in a book that was published in May 2005.[10] An accompanying conference was held in Innsbruck (26–28 May 2005) allowing one to get into contact with the main protagonists in the field of breath analysis.

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