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GC–MS analysis of breath odor compounds in liver patients

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ABSTRACT

Background: Liver diseases can cause a sweet, musty aroma of the breath, called fetor hepaticus. Even in a stage of cirrhosis, the disease can be asymptomatic for many years. Breath analysis might be helpful to detect occult liver pathology.
Study objective: This study examined whether specific breath odor compounds can be found in liver patients, suffering from cirrhosis, which might be useful for diagnosis.
Materials and methods: Fifty-two liver patients and 50 healthy volunteers were enrolled. Alveolar air was analyzed by gas chromatography–mass spectrometry. Using discriminant analysis a model for liver disease was built.
Results: Dimethyl sulfide, acetone, 2-butanone and 2-pentanone were increased in breath of liver patients, while indole and dimethyl selenide were decreased. Sensitivity and specificity of the model were respectively 100% and 70%.
Conclusions: Fetor hepaticus is caused by dimethyl sulfide and to a lower extent by ketones in alveolar air. Breath analysis by GC–MS makes it possible to discriminate patients with breath malodor related to hepatic pathologies.

1. Introduction

Halitosis has a significant socio-economic impact and may reveal disease. It was neglected until recently by scientists and clinicians and is hardly covered in the medical curricula [1].

The vast majority of pathologies causing halitosis lies within the oropharynx (tongue coating, gingivitis, periodontitis, and tonsillitis) and the sulfur containing gases (hydrogen sulfide, methyl mercaptan, and dimethyl sulfide) play a predominant role [2,3]. In 10–15% of the patients, however, breath malodor has an extra-oral cause [4,5]. Examples are foreign bodies in the nose, purulent sinusitis, regurgitation esophagitis and other local factors. Systemic diseases or pathologies distant from the oropharynx, are sometimes revealed by bad smelling metabolites carried by the bloodstream to the lungs. Exhalation of the volatiles that are organoleptically perceived causes halitosis. According to the literature, these extra-oral causes are sometimes associated with a typical odor as a result of specific volatile organic compounds (VOCs) in breath (Table 1) [5–7]. In clinical practice, diabetes mellitus has been associated with the sweet smell of acetone and kidney failure results in a fishy odor. These observations suggest that VOCs in exhaled breath could provide, in a non-invasive way, valuable information about the subjects' pathophysiological condition [6–9].

Liver disease is an important extra-oral cause of bad breath. Patients with various degrees of hepatocellular failure and portosystemic shunting of blood may acquire a sweet, musty or slightly fecal aroma of the breath, termed fetor hepaticus, which has been mainly attributed to sulfur compounds [10]. If the metabolizing function of the liver fails, the concentration of the metabolites, normally processed in the liver, will increase and they will enter again the systemic circulation. Part of them will then be exhaled.

Most patients who complain about breath malodor consult a periodontologist, house doctor or dentist. It is important that clinicians can discriminate liver patients from those with oral malodor. Chronic liver disease, even in a stage of cirrhosis, can be asymptomatic for many years. In this study, gas chromatography–mass spectrometry (GC–MS) was used to examine whether specific odor compounds can be found in breath of liver patients. This could then further be used for differential diagnosis.

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Table 1
Intra- and extra-oral causes of halitosis and their related compounds

Cause	Specific compounds
Oral malodor	Hydrogen sulfide, methyl mercaptan, dimethyl sulfide and dimethyl disulfide
Diabetes mellitus—weight reduction	Acetone, other ketones
Uremia—kidney failure	Dimethylamine, trimethylamine, ammonia
Liver diseases	Dimethyl sulfide, ethanethiol, C2–C5 aliphatic acids (acetic acid, proprionic acid), butyric acid, isobutyric acid, and isovaleric acid
Lung carcinoma	Acetone, 2-butanone, *n*-propanol, aniline, and *o*-toluidine
Upper respiratory/oropharyngeal carcinoma	C2–C8 normal and branched organic acids
Trimethylaminuria	Trimethylamine
Food: garlic/onions	Allyl methyl sulfide
Other potential compounds	Indole, skatole, cadaverine, putrescine, carbon disulfide, and dimethyl selenide

Refs. [5–7].

2. Subjects, materials and methods

2.1. Subject selection

Fifty-two patients (19 females) with liver cirrhosis of various degrees and etiologies (alcohol, medication, hepatitis, primary sclerosing cholangitis, sarcoidosis, primary and biliary cirrhosis) and 50 age-matched healthy volunteers (29 females) were enrolled. All subjects signed informed consent and the research was approved by the Clinical Trials Committee of the University Hospital Leuven. The healthy volunteers were thoroughly questioned on their medical history. All confirmed they were not suffering from any known disease or were not receiving any medical treatment. Nine of them were smokers. Patients with cirrhosis previously confirmed at the hospital by various biochemical and radiological investigations and liver biopsy were selected. 12 of them were smokers. Their MELD-score (Model for End stage Liver Disease), which expresses the degree of liver impairment, ranged from 7 to 40. 35 of them took at least one of the following medications: lactulose, spironolactone, antibiotics, furosemide and propanol. Samples were taken at least 30 min after consumption of any food or beverages, before lunch and at least 2 h after tooth brushing. Volunteers were asked to refrain from eating garlic and onions or any spicy food, 24 h before measurement. They also refrained from drinking alcohol and coffee and to use a mouth rinse 24 h prior to the gas sampling. Fasting was not imposed to avoid the appearance of elevated concentrations of the ketones acetone, 2-pentanone and 2-butanone [11]. Fasting would also have been an impediment for the practical use of this approach. Patients were excluded if they had a history of surgical shunt or transjugular intrahepatic portosystemic shunt (TIPSS), severe chronic obstructive disease or asthma, sedatives or narcotics within the 48 h prior to enrollment, a neurological disorder, Wilson's disease or diabetes mellitus requiring treatment with insuline.

2.2. Sample collection

Sample collection of alveolar air occurred as previously described using a commercial device (Bio-VOC® sampler, Markes International Limited, Rhondda Cynon Taff, UK) [12,13]. Briefly, the following procedure was used. After 60 min rest, the subjects performed a single slow vital capacity breath, into an inert, non-emitting Teflon®-bulb, which has an open end so that the first part of the breath passes through the sampler and only the last portion (150 ml) is trapped. Alveolar air was transferred immediately from the sampler to a sorbent tube to capture all VOCs. This procedure was repeated three times.

The Bio-VOC® sampler was also used to take a sample of room air (same procedure). Two layer sorbent tubes containing 200 mg TenaxTA and 200 mg Unicarb (carbonized molecular sieve) (Markes International Limited) were used. The sorbent tubes were preconditioned with constant flow (90 ml/min) of nitrogen (purity 6.0, a nitrogen purifier: Alltech Associates, Lokeren, Belgium, was used to further increase the purity) using the following temperature program: 1 h at 100 °C, 1 h at 200 °C, 1 h at 300 °C and 30 min at 335 °C. They were then sealed by both Swagelok fitting and PFTE ferrules and stored at 4 °C.

2.3. VOC extraction and analysis

Analysis of samples was performed by GC–MS combined with thermal desorption as previously described [12,13]. VOCs were desorbed and concentrated in a thermal desorber (Unity®, Markes International Limited) at 250 °C onto a −10 °C cold trap for 6 min (helium flow 50 ml/min). The cold trap, packed with the same sorbents as the sorbent tubes, was then heated rapidly to 250 °C and VOCs were transferred to a gas chromatograph (HP6890N, Agilent Technologies, Diegem, Belgium). Column (capillary column, HP5MS, 30 m × 0.25 mm × 0.25 μm film thicknesses, Agilent Technologies) temperatures were ramped as follows: −40 °C for 1 min, 4 °C/min to 180 °C, 0.10 min hold and 30 °C/min to 300 °C, 0.25 min hold. Liquid nitrogen was used as cryogen. Column head pressure of helium carrier gas was set to 10 psi. Purity of helium was at least 6.0 and a helium purifier (Alltech Associates) was used to further increase the purity. Identification of VOCs occurred in a mass spectrometer (HP5973, Agilent Technologies). Mass range was applied from 30 to 350 amu.

2.4. Data management

The presence of all compounds, which have already been associated with halitosis (Table 1), was examined in all breath and environmental samples as previously described [12,13]. Therefore, for each compound an extracted ion chromatogram of the ions, specific for that compound, was made using the Chemstation software (Agilent Technologies). For double-checking, the observed SCAN spectrum was compared with the spectrum in the NIST98 library.

2.5. Quantification

For each compound, detected in at least one breath sample, a calibration curve was made. This procedure has been previously described by Van den Velde et al. [13]. Based on the calibration curve, the concentration of each compound was automatically calculated in both breath and environmental samples. If the compound was also present in the environment, the environmental concentration was subtracted from the concentration in the breath samples [14].

2.6. Statistical analysis

For each compound, a Mann–Whitney *U*-test was performed to detect significant differences between healthy volunteers and liver patients. To correct for multiple testing a Bonferroni correction was included. Forward stepwise discriminant analysis was used to build a model for liver disease. Therefore, volunteers and patients were