universität wien

DIPLOMARBEIT

Titel der Diplomarbeit

"The scent of human diseases:

Specific VOCs as diagnostic biomarkers“

Verfasser

Dr. med. univ. Fanis Buljubasic

angestrebter akademischer Grad

Magister der Pharmazie (Mag. pharm)

Matrikelnummer: 0048460

Studienrichtung lt. Studienblatt: Diplomstudium Pharmazie

Studienkennzahl lt. Studienblatt: A 449

Betreuer: Univ.-Prof. Mag. Pharm. Dr. Gerhard Buchbauer

Wien, 2013

DANKSAGUNG

An dieser Stelle möchte ich mich recht herzlich bei Herrn Univ.-Prof. Mag. Pharm. Dr. Gerhard Buchbauer für die interessante Themenstellung und für die hervorragende wissenschaftliche Betreuung bedanken.

Mein besonderer Dank gilt meinen Eltern, die mir die Ausbildung ermöglicht haben und mir auch in der schwierigen Zeit mit Rat und Tat zur Seite gestanden sind.

Nicht zuletzt bedanke ich mich allen jenen, die ich während meiner Studienzeit in schöner Erinnerung hatte.

ZUSAMMENFASSUNG

Die Anwendung von körperlichen Düften, in Form von flüchtigen organischen Verbindungen (VOCs), in der Diagnose der menschlichen Krankheiten ist seit langer Zeit bekannt und ist in den letzten Jahren zu einem Schwerpunkt der wissenschaftlichen Forschung geworden.

Mehr als 200 VOCs werden aus dem menschlichen Körper freigesetzt, hauptsächlich durch Ausatemluft und liefern dadurch wichtige Informationen über die Stoffwechsellage eines Individuums.

Krankheiten wie Krebs, Infektionen oder Stoffwechselkrankheiten können die Zusammensetzung der täglichen VOCs ändern, was eine Bildung von krankheitsspezifischen VOCs zur Folge hat.

Wenn rechtzeitig erkannt, könnten diese VOCs als diagnostische Biomarker für viele Krankheiten eingesetzt werden.

Demzufolge, wurden in den letzten Jahren viele Studien über die Suche nach krankheitsspezifischen VOCs durchgeführt.

Diese Arbeit ist eine Zusammenfassung von wissenschaftlichen Publikationen der letzten zehn Jahre (2003-2013), die sich mit Studien über VOCs bei menschlichen Krankheiten befassen und somit bereits vorhandene Berichte über die krankheitsspezifische VOCs aktualisieren.

ABSTRACT

The use of body odours, emitted in the form of volatile organic compounds (VOCs), in diagnosing human diseases is known for a long time and has become the focus of scientific research in recent years.

More than 200 VOCs are emitted from the human body, mainly through the exhaled breath reflecting the metabolic condition of individuals.

Diseases such as cancer, metabolic disorders, infections and some other diseases can change the components of daily VOCs, often leading to the production of disease-specific VOCs.

These VOCs might be used as diagnostic biomarkers of many diseases if detected early enough.

Therefore, in the last decade many studies on searching for VOCs, which are specific of certain diseases, have been conducted.

This paper is a summary of scientific publications (2003-2013) related to the study of VOCs in human diseases, thus updating the already existing papers on this subject.

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1. INTRODUCTION

The use of an unusual human smell as a sign of certain diseases dates back to Hippocrates, the father of medicine, to about 400 years BC, who instructed his students to smell the breath of their patients, as well as to pour human sputum on hot coals, in order to produce a smell as a potential indication of human diseases.[1]

In the 11-th century, Arab physician and philosopher Avicenna used his sense of smell in the diagnosis of illness by noting changes in the smell of patients' urine.[2] Traditional Chinese medicine also used the benefit of olfactory medicine for diagnosing diseases, such as diabetes, in patients whose urine had a smell of a rotten apple.[3]

Robert Koch found the connection between foul odours of infected wounds and similar odours of pathogenic bacteria, produced in cultures, with his proof of the germ theory, based on experiments with anthrax in the 19-the century. [4]

These findings were supported by Omelianski, who in 1923, reported on naturally-liberated microbial odours due to the accumulation of staling metabolic products such as organic acids and alcohols in cultures of *Mycobacterium tuberculosis* and *Pseudomonas aeruginosa*. [5]

In the first half of the 1980s a urea breath test was applied in gastroenterology for diagnosing gastritis associated with *Helicobacter pylori*.

Once considered early clues in leading to early diagnosis of diseases, human odours are nowadays analysed by highly sophisticated equipment, such as gas chromatography (GC) with mass spectrometry (GC-MS) and electronic noses. With the help of this equipment, many studies on volatile organic compounds, which represent specific human odours, have been conducted. Identifying those volatile organic compounds may lead to a new era of biomarkers for diagnosing various diseases.[6]

2. VOLATILE ORGANIC COMPOUNDS (VOCs)

Only few non-invasive methods for assessing information on human diseases or monitoring them are available today. For patients with lung diseases, standard screening may include spirometry, plethysmography, lung diffusion testing or radiological investigation (X-ray or computer tomography), for those with gastrointestinal disorders this screening might include stool analysis, breath tests for intestinal disorders (glucose hydrogen breath test for bacterial overgrowth) or ultrasonic investigations. Despite the fact that many patients with lung diseases often complain about a bad scent of their exhaled breath, as well as patients with gastrointestinal diseases, who complain about an unpleasant odour in their faeces, little research has been done to analyse the sudden change in the scent of the exhaled breath or analyse the composition of faecal gases. The same applies to many other diseases, such as infective diseases, metabolic disorders, cancer or even some mental diseases.

Volatile organic compounds are a diverse carbon-based group of chemicals, here emitted from the human body often reflecting the metabolic condition of a person, thus indicating a sudden change in the odour by the abovementioned diseases.

The human body emits every day a variety of VOCs, which are derived from different parts of the body with a specific odour, which varies from person to person. Pathological diseases, genetic disorders or mental diseases might produce new VOCs or cause a change of the odours produced normally.

Nowadays, this could be of great importance in early detection of such diseases.

A physician could for example diagnose a hepatic encephalopathy in patients if he could recognise the change of the odour of patients affected with liver diseases. Therefore, the assessment of VOCs over the past few years has been of interest not only to medical staff, but also to many other scientists in different fields, since VOCs are routinely analysed in assessing contamination of the environment, forensic science or in fragrance industry.

Body odours are emitted daily from the human body and consist of hundreds of VOCs, secreted from the cells as a result of metabolic processes.

They are mostly accumulated in breath, urine, skin, blood, sweat and faeces. Some VOCs are secreted from lung cells and exhaled and some are however secreted into the blood and then emitted to the external environment via breath.

Breath

The VOCs are mainly found in an exhaled breath. However many VOCs come from the external environment and are inhaled regularly by all individuals. Therefore, the composition of VOCs might also reflect the pollution-level of the environment, as well as smoking habits of the patient.

Many VOCs are also emitted from the lungs and exhaled to the environment, which could also be measured at nanomolar or picomolar concentrations. Samples can be obtained easily with the help of non-invasive methods and isolated by GC. Years ago, acetone was isolated from patients with diabetes mellitus, or methylmercaptan from patients with liver diseases causing a “liver breath”. These two VOCs are characteristic of those diseases and could be considered valuable biomarkers for their diagnosis. [7]

Urine

The human urine contains normally various compounds, such as ketones, alcohols, sulphides, pyrroles and many others, which are often end products of metabolic pathways and can be useful for diagnosing human diseases, particularly metabolic disorders. Whereas the scent of urine in patients with trimethylaminuria is only detectable in some cases, acetone or ketones in diabetics are easily detectable. However, according to many studies there has been a significant change in the urine VOCs between cancer patients and normal people. [8]

The scent of urine in patients with bladder infections is popularly called "a uraemic scent" and can easily be recognised.

Urine VOCs are identified by using GC-MS.

Sweat

Fluids secreted by the sweat glands contain a variety of VOCs that can be easily collected. Many VOCs are the result of end metabolism pathway and are excreted directly to the environment via sweat; some are produced due to hormonal changes in the body. However, skin is normally contaminated by bacteria, that are naturally present, and which influence those compounds thus changing the odour of the emitted compounds. The human skin is also a place subject to different bacterial or fungal infections, as well as some metabolic disorders, which all produce a different and in most cases an unpleasant odour. Samples from the sweat /skin can be easily collected by using solid-phase microextraction (SPME) fibre. [9]

Blood

Most VOCs are secreted from cells directly into the blood, as it represents the main means of communication between different parts of the body, collecting the information on the metabolic, nutritional and immunologic status. Due to certain diseases, some compounds may be excreted into the blood causing a change in the odour, which however cannot be detected by human nose. Sniffer dogs have been trained to detect an ovarian cancer in the blood samples of women with ovarian carcinoma in an early stage. Similar reports on lung cancer are also available.
This could have a great diagnostic importance in screening and diagnosing different diseases. [10]

Faeces

Perhaps the earliest and easiest way of diagnosing gastrointestinal and liver diseases is a change of odour in the faeces.
The faeces reflect directly the end of excretory and secretory processes in the organism and are associated with an unpleasant odour.
Some gastrointestinal diseases might cause a change in the odour, such as bacterial infections, pancreatic diseases or cancer. [11]

Although many volatile compounds might be easy to collect from the above-mentioned fluids, they are still influenced by many other factors which could also change the odour. These factors are: sex, age, drug therapy etc.

3. CANCER DISEASES

Lung cancer

Being the leading cause of cancer death, lung cancer has a very poor prognosis, especially when diagnosed in advanced (III and IV) stages.

In most cases, early detection (Stages I and II) of the lung cancer is very difficult, since symptoms characteristic of lung cancer (cough, haemoptysis, dyspnoea, chest pain) often appear only in advanced and metastatic stages and are diagnosed by highly sophisticated equipment (chest radiography, bronchoscopy, sputum histology or computer tomography).

However, in recent years a special attention has been devoted to searching for new biomarkers, which would play a major role in early screening of the lung cancer.

One of these methods might include the analysis of VOCs in exhaled breath of patients with lung cancer. Many studies indicate that specially trained dogs can distinguish normal lung cells from cancer cells, which could play an important role for early detection of lung cancer and reducing the mortality rate, when diagnosed and treated in its early stages.

Furthermore, recent experiments with electronic noses also show promising results in distinguishing the breath of patients with lung cancer from healthy controls which might also play an important role in screening for lung cancer in the future.

All these methods would also have an advantage over the others being non-invasive. In contrast to some other odours related to different diseases, an odour, directly associated with the lung cancer is not perceived. The exhaled breath-samples can be easily collected by using a Teflon bulb. VOCs are isolated by gas chromatography and identified by mass spectrometry.

Different research groups have shown that the exhaled breath of patients with lung cancer differs from the exhaled breath of healthy people.

Phillips et al. [12] published an article in 1999 identifying 22 VOCs in the exhaled breath of patients with lung cancer, 15 of which were either alkanes or alkane derivates and 5 of which were benzene or benzene derivates. The group does not reveal the biochemical pathway of benzene derivates, strongly suggesting that

smoking could not account for the benzene derivates, since those VOCs were obtained from non-smokers or ex-smokers. In addition, the group also suggests, that smoking could not affect the VOCs of lung cancer patients, since 2,5 dimethylfuran, the most common VOC from smoking, was not among the 22 VOCs described in this paper.

This assumption is rather implausible, as only 5 patients out of 60 with lung cancer and 12 out of 48 healthy volunteers had never smoked.

Meanwhile, other studies suggest that most VOCs are not specific of lung cancer and therefore cannot be considered as cancer biomarkers, as they are also present in the exhaled breath of healthy individuals.

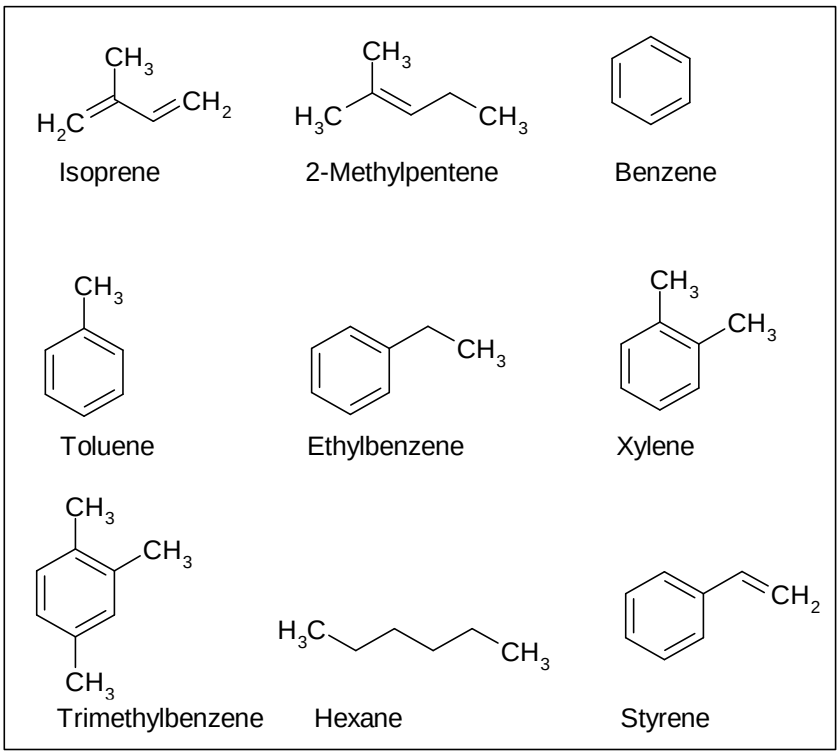


Figure 1: Most common VOCs isolated from patients with lung cancer [12]

Fuchs et al. [13] studied the volatile aldehydes in the exhaled breath of patients with lung cancer, healthy smokers and healthy volunteers.

The results suggest that the concentration of exhaled formaldehyde was significantly lower in the breath of healthy smokers, when compared to patients with lung cancer and healthy volunteers. There was also no difference in the concentration of acetaldehyde, propanal, butanal, heptanal and decanal between the three groups.

However, the exhaled concentrations of pentanal, hexanal, octanal and nonanal were significantly higher in patients with lung cancer compared to concentrations in other two groups. The elevated concentrations of aldehydes are also known in patients with inflammatory diseases. [14, 15, 16] Since oxidative stress has been identified as potential causative agent of tumour genesis, thus having enhanced oxidative activity in tumour tissues, elevated aldehyde concentrations in the exhaled breath of patients with lung cancer are therefore probably generated through oxidative stress resulting in lipid peroxidation of the cell membrane.

In this context, specific aldehydes may be produced during lipid peroxidation if specific unsaturated fatty acids are present in tumour cell membranes, thus revealing the biochemical pathway of these VOCs, released in the exhaled breath of patients with lung cancer. (Figure 2)

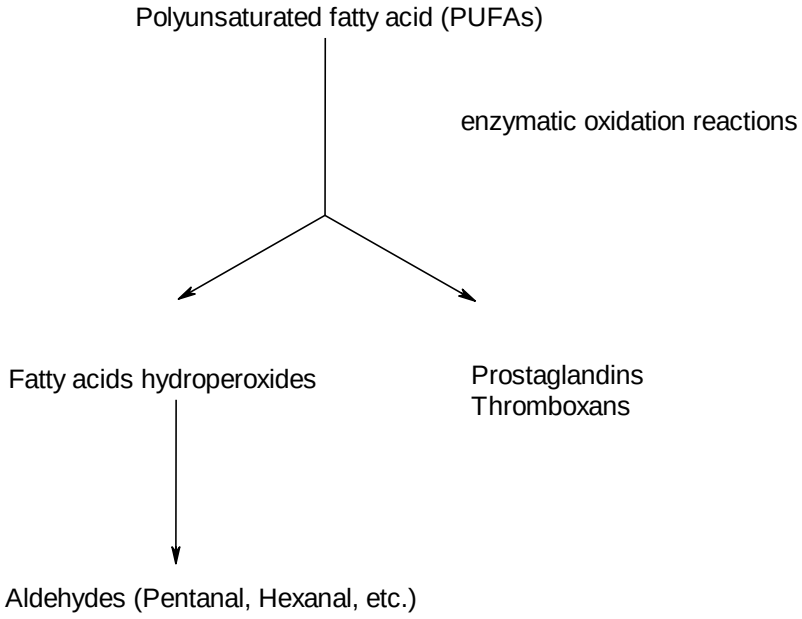


Figure 2: Polyunsaturated fatty acid products [17]

Poli et al. [18] isolated the VOCs from:

1.) Patients with lung cancer in all stages

2.) Smokers without any cancer

3.) Healthy non-smokers

4.) Patients with chronic pulmonary disease (COPD)

In this study a total of 13 VOCs were isolated from all 4 groups.

The first group of patients underwent a surgical removal of the lung cancer and while assembled for the follow-up 3 years later, the exhaled breath-samples were also collected from them.

In these patients the amount of isoprene and benzene was significantly higher in the exhaled breath compared to the healthy persons.

Three years later during the follow-up after a surgical removal of the cancerogenous tissue, the amount of isoprene and decane was significantly lower after the surgery. The amount of other VOCs was not significantly different before and after surgery. For this reason, these 3 VOCs might be used as future biomarkers for the follow- up.

However, the amount of isoprene was not only higher in patients with lung cancer before surgery, but also in healthy smokers, when both groups were compared to patients with chronic pulmonary disease, which indicates that isoprene might be a good biomarker only for the follow-up.

Isoprene is generated along the mevalonic pathway of cholesterol synthesis in the cytosolic fraction. (Figure 3) [19]

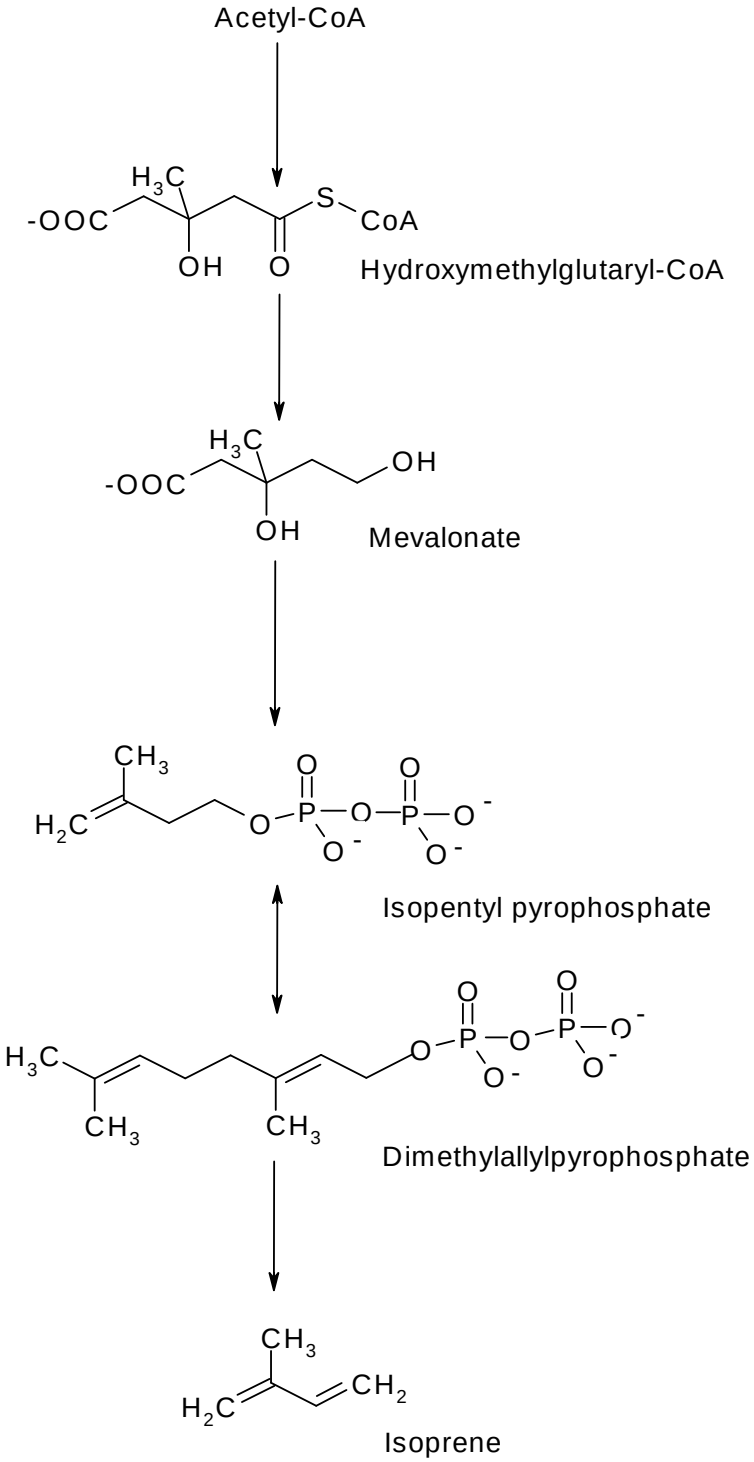


Figure 3: Biochemical pathway of isoprene generation [19]

There is a discussion, that isoprene may also be released during the oxidative stress. Why in patients with lung cancer the isoprene-level is higher than in healthy persons still remains unclear.

Furthermore, the concentration of two other VOCs (pentane and 2-methylpentane) was also higher in the exhaled breath of patients with lung cancer.

Whereas pentane was not only higher in patients with lung cancer but also in healthy smokers, 2-methylpentane was significantly higher only in patients with lung cancer, thus being the most specific biomarker in the exhaled breath of patients with lung cancer.

Whereas the origin of 2-methylpentane is still debated, [20] pentane like most other hydrocarbons are markers of lipid peroxidation of polyunsaturated fatty acids (PUFAs) found in cellular membranes. [21] Lipid peroxidation is a free radical-mediated process, where PUFAs are affected, leading to the formation of a variety of carbonyl secondary oxidation products. Thanks to their bond energies, PUFAs are susceptible to hydrogen abstraction reactions (LOO°), that initiate or propagate an autooxidation process. (Figure 4)

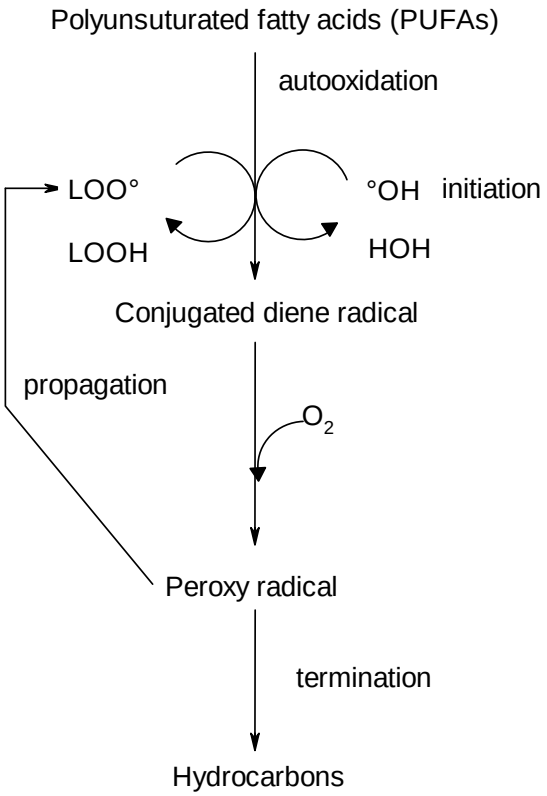


Figure 4: Biochemical pathway of hydrocarbons (e.g. pentane) [17]

Slightly elevated levels of pentane are common among smokers, as smoking also causes lipid peroxidation, which explains why healthy smokers also showed elevated levels of pentane in the exhaled breath.

Although this study could identify 3 more VOCs, none of them was specific of lung cancer. The advantage of the study might be the recruitment of healthy non-smokers, who had decreased levels of pentane in the exhaled breath, compared to lung cancer patients, patients with COPD and healthy smokers. Still, elevated pentane levels could not differentiate the last three groups from each other.

Studies have also been made in trying to predict lung cancer using volatile biomarkers in breath. [21]

Two groups of patients were included: patients who had lung cancer diagnosed including its early stages and patients without signs of lung cancer after a chest-computer tomography was performed. The collected breath was analysed by GC-MS, which led to the identification of 16 VOCs, which attained higher concentrations in patients with lung cancer, when compared with healthy controls. These results suggest that certain VOCs might be used for lung cancer-screening in its early stage.

The control of these VOCs in the exhaled breath may also be used for the follow-up in patients after surgical removal of the lung cancer or after being subjected to chemotherapy. It is not sufficient to control only one substance, but rather a combination of them. [22, 23]

There are also 3 publications about the VOCs released in-vitro by different lung cancer cell-lines, such as CALU-1. [24] The VOCs were emitted and isolated from cancer cells before incubation, 4 hours and 18 hours after incubation. The VOCs of the CALU-1 cells were isolated and measured by GC-MS.

The results suggest that the amount of: 2,3,3-trimethylpentane, 2,3,5-trimethylhexane, 2,4-dimethylheptane and 4-methyloctane was significantly higher and they might be considered as cancer-cell-derived VOCs, indicating the presence of a tumour. On the other hand, decreased concentrations of: acetaldehyde, 3-methylbutanal, butyl acetate, acetonitrile, acrolein, methacrolein, 2-methylpropanal, 2-butanone, 2-methoxy-2-methylpropane, 2-ethoxy-2-methylpropane and hexanal were found, which might indicate that other VOCs were consumed by CALU-1 cells.

One of the possible explanations for decreased concentrations of acetaldehyde might be an increased activity of alcohol dehydrogenase in tumour cells.

These findings are contradictory to the work of Smith et al. [25], whose work describes increased concentrations of acetaldehyde in CALU-1 and SK-MES, also a lung cancer cell line. The reasons for different concentrations of acetaldehyde in CALU-1 cells are unknown.

Another publication [26] dealing with the VOCs exhaled by lung cancer cells in vitro shows that 4 VOCs of several lung cancer cells were significantly increased after being cultured together with bronchial epithelial cells, tastebud cells, osteogenic cells and lipocytes later identified by gas chromatography. The VOCs released by lung cancer cells were: styrene, isoprene, decane and benzene.

Furthermore, the authors tried to find a correlation between those 4 VOCs released by lung cancer cells and the VOCs in the exhaled breath of patients with lung cancer in stages I and II. Their findings also suggest that the same VOCs were detectable in patients with lung cancer with stage I and stage II. As those VOCs were normally found in the exhaled breath of patients with lung cancer in advanced stages (III and IV), these results might lead to the development of new methods of non-invasive screening for lung cancer in its early stages, which are very difficult to diagnose even by using a highly sophisticated equipment (x-ray, CT, bronchoscopy).

Numerous studies also indicate that dogs with a much more developed olfactory system can sniff many cancer types, especially the lung cancer. [27] As mentioned earlier, an odour characteristic of lung cancer is not perceived, contrary to some other diseases (e.g. fish-like scent in patients with trimethylaminuria). In the past few years, dogs have been trained to distinguish the breath of patients with lung cancer from the breath of healthy persons. They were first trained to sniff the breath samples of patients suffering from lung cancer in all 4 stages by spending some time with the patients, as well as being in company with healthy persons. Once trained, they were brought to the breath samples of the patients with lung cancer, who they had previously never seen. Sitting or lying in front of the samples of the patients with lung cancer was a sign of recognising cancer, whereas ignoring the samples was a sign of sniffing the breath sample of healthy persons. Canine scent detection of lung cancer was accurate (95 -98 %). [28]

Perhaps, the most striking result was, that dogs were able to detect the lung cancer in its early stages (I and II) which could be of great importance for early detection of the lung cancer, as patients with this cancer do not show early symptoms.

Sniffer dogs could be useful for this purpose as another non-invasive method of screening, but many technical problems arise when dealing with the training of dogs, as well as building special labs for them inside hospitals. [29]

Another important non-invasive screening tool for lung cancer might include electronic noses, which have been in rapid use since 1990. [30]

They are popularly called “artificial olfaction systems” and are able to recognize, identify, and classify gaseous samples as the human olfaction does with odours.[31]

They are arrays of non-selective solid-state sensors whose response is not univocally correlated with the concentration of a single compound but it is a sort of combination of the chemical information contained in the sample, thus encoding the global composition of a sample into a pattern of sensor signals. This mode of operation can be compared with the principle of natural olfaction where applying a sort of combinatorial selectivity, some hundreds of different receptors enable humans to distinguish among tens of thousands of different odours. [32]

In recent years these arrays of chemical sensors were applied in many different fields providing in many cases useful identification and classification of samples from health care sectors. [33]

Amico et al. [30] studied the use of electronic noses to distinguish patients with lung cancer from other lung diseases and healthy controls.

In their study 3 groups of patients were included: patients with different stages of lung cancer, patients with no history of cancer but with other lung diseases such as chronic obstructive pulmonary disease and healthy volunteers. The results are promising, as the electronic noses could distinguish breath samples of patients with lung cancer from other diseases and healthy controls with a sensitivity of 85 % and a specificity of 100 %. These results are supported by Dragonieri et al. [34], who studied the application of electronic noses in distinguishing the breath samples of patients with lung cancer from patients with chronic obstructive pulmonary disease and from healthy controls.

Their results suggest that VOC-patterns of exhaled breath discriminate patients with lung cancer from COPD patients with an accuracy of 85 %, as well as from healthy controls with an accuracy of 90 %.

Malignant mesothelioma is a rare cancer which affects the membrane lining (mesothelioma) of the lungs and abdomen. Chapman et al.[35] used a carbon polymer array, which is considered a most reliable array in distinguishing breath profiles of patients with lung cancer from healthy controls, in their experiments with the breath samples from patients with mesothelioma, patients with other respiratory diseases (asbestos-related diseases) and healthy controls.
Smell prints of 10 patients with malignant mesothelioma were used as a training set. Smell prints from 10 new patients with this tumour were distinguished from control subjects with an accuracy of 95%. For smell print identification between malignant mesothelioma, asbestos-related diseases and control subjects the electronic nose had a sensitivity of 90% and a specificity of 88%.

Dragonieri et al. [36] used an electronic nose, composed of chemical vapour analyzer, containing a nanocomposite array with 32 polymer sensors for the same purpose with 13 patients with malignant mesothelioma, 13 patients with asbestos-related diseases and 13 healthy subjects. In their publication breath prints from patients with malignant mesothelioma were separated from patients with asbestos-related diseases with an accuracy of 80 %. Breath prints from patients with malignant mesothelioma were separated from healthy subjects with an accuracy of 84 %.
Recently, more sensitive and developed electronic noses, called “NA-NOSE” have been in use, as well. The electronic nose “NA-NOSE” is a nanoscale artificial nose, based on an array of highly cross-reactive gas sensors, mainly chemiresistors based on different monolayer-capped metal nanoparticles) that can identify and separate different odours, even if they are present at very low concentrations. Each sensor shows an individual response to all (or to a certain subset) of the volatile biomarkers that make up the cancer-odour. The odour is identified by analyzing the sensor signals with a statistical pattern recognition method. [37]
Barash et al. [38] reported on the application of electronic nose with gold nanoparticles in detecting a "unique odour" of non-small-cell -lung-cancer cells. This electronic nose could distinguish a breath pattern of patients with this cancer from healthy controls with 100 % accuracy at very low concentrations.

The use of an electronic nose for detection of lung cancer offers several potential advantages but disadvantages as well. Its advantages are high sensitivity and portability of the detector. Its disadvantages are loss of sensitivity in the presence of water vapour or high concentrations of a single component, sensor drift and the inability to provide absolute calibration, relatively short life of some sensors and the inability to obtain quantitative data. Still, the use of NA-NOSE in the last few years shows more promising results.

Breast cancer

Being the most common cancer of women after skin cancer and originating from breast tissue, two types of breast cancer are known: the cancer of milk ducts and the cancer of lobule. [39]

In the past few years there has been a significant progress in treating breast cancer, thus increasing an overall 5-year survival rate up to 84 %, when diagnosed in the early stage. The available screening tests include: mammography, ultrasound and magnetic resonance imaging. [40]

Still, diagnosing breast cancer in the early stage occurs rarely due to the lack of early symptoms. [41]

Breast cancer is known to be accompanied by an increased oxidative stress, as well as by an induction of polymorphic cytochrome 450- mixed oxidase enzymes, during which a large number of VOCs are produced and excreted in the breath. Phillips and his group [42] studied these VOCs excreted by women with breast cancer and analysed them by GC-MS.

Three groups of women were included in this study:

1. Women with positive histological evidence of breast cancer
2. Women with negative histological evidence of breast cancer
3. Healthy volunteers

This group used a more extensive set of breath markers than pentane alone, called "breath-methylated–alkane-contour" (BMAC), which is a three-dimensional display of the alveolar gradients of C4–C20 alkanes and monomethylated alkanes. The following VOCs could be detected in a higher concentration in women with breast cancer, when compared to the healthy controls: nonane, 5-methyl tridecane, 3-methyl undecane, 2-methyl propane, 6-methyl pentadecane, 4-methyl dodecane and 2-methyl octane

Seven years later the same group [43] identified more VOCs characteristic of breast cancer. This time the three groups of women were different from the three groups in the first study and they included:

1. Women with positive histological evidence of breast cancer
2. Women with abnormal mammography but without positive histology
3. Healthy volunteers with neither positive histology nor abnormal mammography.

Breath samples were collected by a portable breath collection apparatus. The VOCs were collected before biopsy had been done and after collection they were isolated and identified by gas chromatography and mass spectrometry. Similar to lung cancer a characteristic scent of breast cancer is not perceived. The results showed that at least 10 VOCs could be identified, which were found to be increased in women with breast cancer compared with healthy volunteers. It was already known that pentane levels were increased in patients with breast cancer, but pentane was also increased in patients with rheumatoid arthritis, asthma bronchiale and schizophrenia.

The biological mechanism of production of VOCs of the breast cancer is speculative. Many studies suggest that breath biomarkers of other cancer types were generated by accelerated catabolism of normal metabolic products, consistent with cancer-associated induction of cytochrome P450 enzymes. [44]

The group proposed a hypothesis, based on the altered metabolism of estrogen that may account for the volatile biomarkers in the breath of patients with breast cancer due to the activation of cytochrome-450-system, which comprises a group

of inducible mixed-function oxidase enzymes that metabolize drugs and the VOCs produced by oxidative stress. This enzyme is normally present in breast tissue and is activated in cancer tissue (aromatase). Activation of aromatase results in accelerated biosynthesis of estrogen with increased risk of breast cancer. (Fig. 5) The increased cytochrome P450 activity associated with breast cancer may modulate the composition of VOCs excreted in the breath of patients with breast cancer.

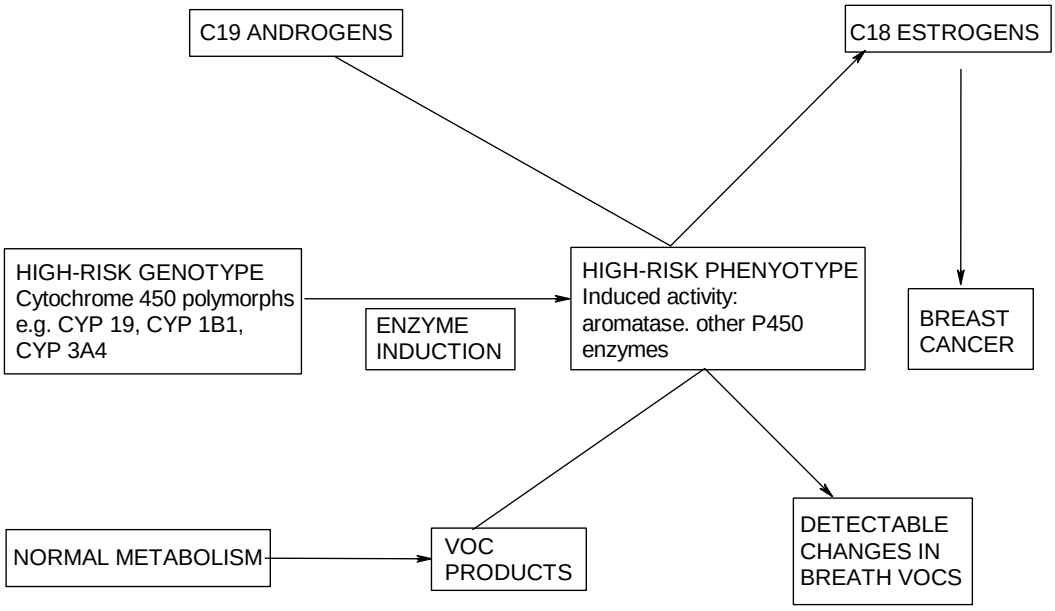


Figure 5: Hypothetical basis of the breath test for breast cancer [43]

As mentioned before, alkanes and methylated alkanes are known to be markers of oxidative stress.[45] The above mentioned VOCs are also excreted in the human breath due to oxidative stress and are degradation products of membrane PUFAs, which have undergone lipid peroxidation by reactive oxygen species liberated from mitochondria and since oxidative stress plays a major role in breast cancer, the increased abundance of those VOCs is related to the activation of oxidative stress in women with breast cancer. [44, 46, 47, 48]

The origin of VOCs is not known either and since VOCs, such as pentane are also increased in patients with lung cancer, studies have shown that the level of this VOC after surgical removal of cancerogenous tissue was lower, which also might suggest, that it is produced in the lungs.

The weakness of this study is a lack of specificity. The authors did not mention if the recruited volunteers had other diseases (asthma bronchiale, rheumatoide arthritis and schizophrenia) associated with oxidative stress and which are also characterised by elevated pentane levels.

Therefore, elevated pentane levels in this study cannot only be attributed to the oxidative stress in breast cancer cells.

The first application of NA-NOSE in detection of breast cancer was investigated by Peng and his team in 2010. [49] Exhaled breath patterns were collected from 177 volunteers, who included: patients with lung cancer, patients with breast cancer, patients with colorectal cancer, patients with prostate cancer and healthy controls.

The results suggest that NA-NOSE distinguished the exhaled breath of healthy persons from the exhaled breath of patients with different types of cancer. Moreover, it could also differentiate between the breath patterns of different cancers, irrespective of age, gender or life style.

The 5 most common VOCs, unique of breast cancer were:

1. 3, 3-Dimethyl pentane
2. 2-Amino-5-isopropyl-8-methyl-1-azulene carbonitrile
3. 5-(2-Methylpropyl) nonane
4. 2, 3, 4-Trimethyl decane
5. 6-Ethyl-3-octyl ester 2-trifluoromethyl benzoic acid

Schuster et al. [37] further applied the use of NA-NOSE in detection of breast cancer precursors in the exhaled breath. The NA-NOSE could not only distinguish the exhaled breath of women with breast cancer from healthy controls, but also the exhaled breath of different benign breast conditions (negative mammography and negative biopsy) from breast cancer and healthy controls which might suggest, that also benign breast conditions have a unique breath pattern when analysed by NA-NOSE. However, the group did not mention the VOCs, characteristic of those benign breast conditions.

Although, many more information on the application of NA-NOSE in detection of cancer are still needed, the first results show that NA-NOSE might have a slight advantage over GC-MS, since the latter suggests, that each cancer could have a unique pattern of VOCs when compared with healthy states, but not when compared with other cancer types. On the other hand, NA-NOSE is capable of differentiating the breath patterns between different cancer types.

Women with abnormal mammography and with no histological evidence of cancer also had an increased abundance of the above mentioned VOCs in the exhaled breath. This can be easily explained by the excessive activation of oxidative stress in these women or maybe due to higher age, as oxidative stress is increasingly activated in higher age.

Since the breath tests can accurately separate women with breath cancer from healthy volunteers, it might be used as a future screening method for excluding breast cancer. The standard screening tools like mammography or magnetic resonance imaging may either be too painful or too expensive to use. It is even more important to stress that mammography is forbidden in some countries if typical symptoms suspicious of breast cancer are not present due to a radiation exposure of breast tissue, which is very sensitive to radiation and might also become precancerous. This means, that a woman with a negative breath-testing should not be subjected to the standard screening method, as the accuracy of breath testing is equal to that of mammography and much cheaper, as well.

Still, many more data and studies have to be done and analysed, before this method could become a diagnostic routine.

However, the available data suggest, that it is a promising tool for early screening of breast cancer.

Cancer of urinary tract

Two most common cancer types of the urinary tract are bladder cancer and prostate cancer, the latter being even the most frequent malignancy in men. [50]
In the past decade, using a prostate specific antigen (PSA) in prostate cancer screening has become a standard method of selecting patients for a biopsy. [51]
Sarcosin as another prostate cancer marker, has been more useful in screening as a marker for aggressiveness (metastatic prostate cancer), as well as for distinguishing a benign prostate hypertrophy from prostate cancer. [52]
However, both PSA and sarcosine show a lack of specificity, both being significantly increased in some other diseases as well. [53]

Interestingly, only two studies describing a total of 6 VOCs released in patients with cancer of urinary tract have been published until now.
Therefore, many more studies will have to be conducted before we open a discussion on the VOCs specific of this type of cancer.

Spane and his group [54] suggest that patients with prostate cancer had an elevated concentration of formaldehyde in the urine which might also include blood and breath of these patients.
The VOCs were detected in 18 patients, who were mostly in the first two stages of prostate cancer, which might be a reason why a significant abundance of formaldehyde was not detected in the exhaled breath. [54]
However, this is important in detecting the VOCs in the early stages of prostate cancer, in which a PSA is not always significantly elevated in the blood samples and most clinical symptoms are not present.
Cornu et al. [55] evaluated the efficiency of prostate cancer detection by using sniffer dogs in distinguishing the urine odour of patients with prostate cancer from healthy volunteers.
As of now, no extensive studies on the VOCs in the urine, related to the prostate cancer have been published.
Based on the data of canine detection of lung cancer, a Belgium malinous shepherd was trained for 24 months to recognise the urine samples of PC-patients and the urine of healthy volunteers by a clicker method (a kind of operant

conditioning). The dog was trained to sit in front of the cancer urine and each time upon completing his task he was given a ball as a reward.

The patients, who had an elevated PSA in the blood and after undergoing a biopsy, were included in this study only after having a positive biopsy. A total of 108 supplied urine and 66 patients were tested in a double blinded study. All urine samples were frozen for storing and heated before testing.

The double-blind testing comprised consecutive runs. For each run, the dog was presented with six samples (five controls and one cancer). During each run, the cancer urine was one of the 33 selected cancer samples and the 5 control urines were samples randomly selected among controls. People who were conducting the test were not able to discriminate cancer from control samples.

During each run, the dog had to scent successively the six samples that were hidden in boxes through a hole. After half a minute the dog had to sit in front of the box, indicating that he designated the cancer sample. This result was classified as a true positive and the controls as true negatives, after the dog ignored the control sample. In case of mistake (dog sitting in front of control urine sample), the control sample was classified as false positive and the cancer sample as a false negative. The false-positive sample was excluded from the pool of controls used for the future runs, and the cancer sample was retested in association with other controls.

A total of 33 runs were conducted during the double-blind testing phase.

In 30 cases, the dog sat in front of the cancer sample. In 3 runs, the dog sat in front of a control sample and in these 3 cases the control samples incorrectly classified were considered false positives, whereas the three cancer cases were considered false negatives. Consequently, during the testing phase, the dog correctly classified 60 samples out of 66. In the three cases where the dog failed the run was conducted again using the same cancer sample and other control samples. The dog classified cancer samples as true. The three patients, whose urine was falsely classified as positive, underwent a new biopsy. One of them was diagnosed with a prostate cancer. This study might suggest, that there are odour signatures characteristic of prostate cancer in the urine, similar to the unique odour signature in the exhaled breath of patients with prostate cancer.

However, this study describes only one trained dog presented with urine samples of cancer patients, many of whom were >50 years. It is unknown if similar results could be obtained from younger patients and also by training other dogs.
As mentioned above, until now only two VOCs (sarcosin and formaldehyde) related to prostate cancer have been isolated from the urine in an increased abundance. The urine samples in this study were frozen upon collection and again heated before testing, which might also result in a loss of many VOCs from the urine before being subjected to dog sniffing. After all, their quantity also correlates with a different stage of this disease, indicating that sniffer dogs might only be able to recognise the urine of the patients with large quantities of the VOCs characteristic of prostate cancer.
This study is the first step of VOC detection for diagnosing prostate cancer and this approach should be continued by determining the volatile molecular signature of prostate cancer in the urine.

Peng and his group [49] also isolated the VOCs from patients with prostate cancer in the exhaled breath by using GC-MS.
The VOCs detected in patients with prostate cancer include:

1. 2–Amino -5 isopropyl-8-methyl-1-azulene carbonitrile
2. Toluene
3. p-Xylene
4. 2, 2 -Dimethyl decane

However, the authors do not speculate on the origin of the VOCs presented in this study.
Since most aromatic hydrocarbons and branched-alkanes are environmental biomarkers, the authors should be cautious about classifying the abovementioned VOCs as the ones specific of prostate cancer.
In the same study, they could also distinguish between the patterns of four different types of cancer (lung cancer, breast cancer, colorectal cancer and prostate cancer) by using NA-NOSE.
Still, this study could only present a proof of concept, since a very small test population was used.

The bladder cancer is derived from an epithelium and most patients with blood in their urine, pain during urination and other signs and symptoms suspicious of a bladder cancer, are referred to a urologist for a cystoscopy, a procedure in which a flexible tube camera is introduced into the bladder through the urethra, thus making it possible for suspicious lesions to be biopsied. However, this standard method in diagnosing the bladder cancer is invasive, costly and depends largely on physicians' experience. [56]

Willis et al. [57] published her study on canine detection of bladder cancer by sniffing urine samples. The aim of her study was to determine if dogs were able to recognise the patients with bladder cancer on the basis of urine scent more successfully than it would be expected by chance alone.

A total of 106 diseased and healthy controls supplied urine. All patients viewed as positive controls underwent a cytoscopy, as well as a biopsy, which detected the cancer cells. The healthy controls used in this study had no positive history of any malignant diseases. Some other diseases in connection to the healthy patients, such as diabetes, chronic obstructive pulmonary diseases or cardiovascular diseases were not exclusion criteria.

The dogs were trained for 7 months by sniffing dried urine samples of the healthy controls. In the testing phase dogs were presented with 7 different urine samples, one of which was the cancer urine sample and they were trained to lie by the urine sample with bladder cancer. After the test run, the results were analysed by a T-test to determine the probability of detecting the urine samples of the cancer patients.

The results suggest that dogs recognised 22 out of 54 samples correctly (41%) compared with 14% expected by chance. The dogs trained to sniff the wet samples were able to recognise the cancer samples better then those trained to sniff the dried samples. This gives us again an assumption, that many VOCs are lost in the drying process or that at least a large part of them may be lost after the drying procedure.

However, the main purpose of this study was only to determine if dogs were able to recognise the urine samples of the patients with bladder cancer, which would indicate that the bladder cancer also has a unique odour print in the urine.

As already mentioned, they could recognise the cancer samples with an accuracy of 41%, when a T-test is applied, which is still better when compared with a chance alone (14 %).
Unlike prostate cancer, no VOCs characteristic of the bladder cancer, except formaldehyde have been identified yet. For this reason, it was also very difficult to train the dogs, as there was no specific odour, which is related to this cancer and that would also serve as a positive control. [57]
Still, this study has paved the way for identifying the specific VOCs from the urine and also for further experiments with dogs.

Ovarian Cancer

An ovarian cancer accounts for about 5 % of all cancers in women with a high mortality rate, due to the fact, that this cancer is in every second case diagnosed in advanced stages.
However, if diagnosed in its early stages, it also represents one of the best curable cancers. Therefore, many attempts have been made to develop new screening methods for diagnosing this disease, since it is very difficult to diagnose it in its early stages (I and II) due to a lack of early symptoms. [58]
The screening methods for this cancer in high-risk patients include ultrasound imaging and serum biomarker CA-125.
Although ultrasound scanning can be adopted for widespread screening, [59] the use of tumour marker CA-125 lacks its sensitivity for detecting early stages of ovarian cancer and its specificity, since elevated levels of this biomarker are also found in individuals without ovarian cancer. [60]

Horvath et al. published the results of three studies, in which they used dogs and electronic noses in detecting an ovarian carcinoma. In the first study samples from different cancerogenous tissues were examined, as well as normal tissue from healthy women by a single dog. [10] The trained dog could distinguish the odour of different gynecological malignancies including benign tumours from the odour of control samples with a sensitivity of 100 % and a specificity of 97, 5 %.

It is not only interesting, that the dog could recognise the cancer samples with such a high sensitivity, but he also managed to distinguish the cancer samples from the samples with other gynecological problems, as well as, to recognise the cancer samples in the early stages. This might suggest that an ovarian carcinoma has its unique odour print in the blood which is also present in the early stage of this cancer. [10]

Two years later, the same group conducted a study on electronic noses in distinguishing the odour of different gynecological tumours from the odour of healthy tissue with a sensitivity of 84, 4 % and a specificity of 86, 8 %.

Both sensitivity and specificity were lower when compared to the previous study with canine detection. [61]

The third study included again sniffer dogs in distinguishing the blood odour of women with an ovarian cancer from the blood odour of healthy women.

Tissue samples were collected from women aged 35-79 and blood samples from women aged 45-77, who had a positive biopsy and the cancer was also present in all stages and of different histological types.

The healthy controls had a negative biopsy, but a few of them still had some other gynecological problems. Two dogs were trained for 9 months to sniff the cancer tissue and after showing an interest, it was suddenly snatched away.

The tests were conducted according to the double-blind study with a positive response with a dog scratching the sample or lying down in front of it. The dog identified correctly all cancer samples, giving a sensitivity of 100%.

Two controls out of 50 were indicated, giving a specificity of 98 %. [62]

Since there is no accepted screening method for diagnosing an ovarian cancer, the use of sniffer dogs with a very high specificity in sniffing the cancer samples even in its early stages might be a most promising non-invasive screening method. However, the same problems arise again when the costs, space and training sniffer dogs are taken into consideration.

Future studies will also concentrate on the application and development of electronic noses for screening of ovarian carcinoma by testing the blood/plasma.

Colorectal cancer

The colorectal cancer is the third most common cancer in both men and women. Colonoscopy with a biopsy remains the main screening method for diagnosing this cancer. [63]

Peng et al. [49] investigated the ability of a nanosensor array to discriminate between breath VOCs of different cancer types (lung cancer, prostate cancer, breast cancer and colorectal cancer). The breath from healthy controls and cancer patients was examined by a tailor-made array of cross-reactive nanosensors based on organically functionalised gold nanoparticles and gas chromatography linked to the mass spectrometry technique (GC-MS). The exhaled breath of patients with colorectal cancer contained high levels of 6 different VOCs when compared to healthy controls and other 3 cancer types, which might present a typical odour signature of colorectal cancer in the exhaled breath.

These VOCs are:

1.) 1, 10-(1-Butenylidene)bis benzene

2.) 1, 3-Dimethyl benzene

3.) 1-Iodo nonane

4.) 1, 1-Dimethylethylthio acetic acid

5.) 4-(4-Propylcyclohexyl)-40-cyano [1, 10-biphenyl]-4-yl ester benzoic acid

6.) 2-Amino-5-isopropyl-8-methyl-1-azulene carbonitrile

However, there was no mention of the origin and metabolic pathway of the above mentioned VOCs in this study.

Silva et al. [64] investigated the urinary VOCs as potential cancer biomarkers by solid-phase microextraction in combination with gas chromatography-mass spectrometry. Urine samples of 33 cancer patients (colorectal cancer, lymphoma, and leukaemia) and 21 urine samples from healthy controls were used in this study. A total of 82 VOCs were identified, of which 5 were found to be significantly higher in patients with colorectal cancer, compared to healthy controls, lymphoma and leukaemia patients.

These VOCs are:

1.) Anisole
2.) 4-Methyl-2-heptane
3.) Hexanal
4.) 3-Heptanone
5.) 1, 4, 5- Trimethyl-naphthalene

The origin and metabolic pathway of these VOCs were not given in this publication, either.
Until now only 3 publications dealing with colorectal cancer have been published. The last one was published by Sonoda et al. [65] in which they described, similar to the other publications, sniffer dogs detecting colorectal cancer.
In their study they used a trained Labrador dog for distinguishing watery faeces of patients with colorectal cancer from healthy controls. Their results show, that the dog managed to recognize watery faeces of patients with colorectal cancer with a sensitivity of 97 % and a specificity of 99 %, which is even superior to colonoscopy (sensitivity 91 % and specificity 99 %).
The dog was also able to recognize the samples of colorectal cancer even in its early stages, which indicate that a specific colorectal cancer scent does exist.

Head-neck cancer

The head-neck cancer represents the 8-th most common malignancy in the world and has very limited therapeutic options if not diagnosed in its early stage. [66]
Hakim et al. [67] investigated the use of a tailor-made artificial nose based on 5 gold particle sensors in analyzing the exhaled breath of 87 volunteers, who included patients with lung cancer, patients with head-neck cancer and healthy controls.
The VOCs specific of head-neck cancer, thus distinguishing the cancer from healthy controls and which were identified with NA-NOSE and GC-MS are:
1.) 4, 6-Dimethyl-dodecane
2.) 2, 2-Dimethyl-propanoic acid

3.) 5-Methyl-3-hexanone

4.) 2, 2-Dimethyl-decane

5.) Limonene

6.) 2, 2, 3-Trimethyl-exobicyclo [2.2.1] heptane

The VOCS distinguishing the head-neck cancer from the lung cancer were:

1.) Ammonium acetate

2.) 3-Methyl-hexane

3.) 2, 4-Dimethyl-heptane

4.) 4-Methyl-octane

5.) p-Xylene

6.) 2, 6, 6-Trimethyl-octane

7.) 3-Methyl-nonane

The results show that NA–NOSE could clearly distinguish between cancer patients and healthy controls and was superior to GC-MS in distinguishing head–neck cancer from lung cancer, as well as from healthy controls.

The results of this study suggest that one could develop a cost effective, fast and good method for diagnosing head-neck cancer with breath testing by using NA-NOSE as a screening tool, since this cancer is unfortunately often diagnosed in advanced stages.

Although the authors acknowledge, that the abovementioned branched alkanes might be products of lipid peroxidation, they surprisingly avoid discussions about the metabolic origin of the branched alkanes in their study.

One more publication dealing with this subject was published by Schmutzhard et al. [68], in which they tested the exhaled breath of patients with head-neck cancer for the first time with a proton transfer reaction-mass spectrometry in order to establish a minimal invasive screening method.

The results show that the patients with cancer had elevated acetonitrile and isoprene levels, compared to healthy controls.

Still, further research has to be done before accepting those two VOCs as “odour signature” for the head-neck cancer, since isoprene levels might also be elevated due to bacterial superinfection or activation of the immune system. In almost the

same manner, acetonitrile levels are sometimes elevated by smokers without any cancer diseases.

Other malignancies

Qin et al. [69] reported on the exhaled breath samples of patients with hepatocellular carcinoma and analysed them with GC-MS. Their results show that patients with this cancer had elevated levels of: hydroxy-2-butanone, styrene and decane compared to healthy controls.
Williams et al. [70] published an article on the canine scent detection of melanoma. However, more studies on both malignancies have to be conducted in the future before identifying the VOCs characteristic of them.

4. GENETIC AND METABOLIC DISORDERS

Most genetic disorders are characterised by either enzyme deficiencies or transport defects, which causes the excessive accumulation of metabolites mostly in blood and urine, but in certain diseases also in breath and other body fluids. Due to a lack or improper function of these enzymes abnormalities in normal metabolic pathways follow. The elevated levels of those metabolites lead to the diagnosis of those diseases, most of which are lethal if not treated at a very early stage.

Moreover, these disorders are often associated with a specific odour and are therefore considered first signs in diagnosing the genetic disorders in countries, in which neonatal screening tests are still not available. If the metabolites are accumulated in body fluids in large amounts, clinicians could diagnose them easily by their specific odour.

Phenylketonuria

Phenylketonuria is an inherited recessive autosomal disease with high levels of the amino acid phenylalanine in the blood, due to a deficiency of enzyme phenylalanine hydroxylase which converts it into tyrosine.

As a result of this deficiency the amino acid phenylalanine is accumulated and later metabolised into phenylpyruvic acid and phenylacetate (Figure 6), which are both harmful to the central nervous system and might cause brain damage.

Although phenylketonuria is nowadays included in the newborn screening, this disease was recognisable to physicians by its unique smell. The children with phenylketonuria have a mousy or barny smell.

In the past parents also noticed a musty odour in the sweat and urine of their children and they also complained of the odour, similar to the smell of locker rooms which later led to the diagnosis of this disease in the hospital. The intensity of the smell is related to the concentration of phenylacetate in the urine. [71]

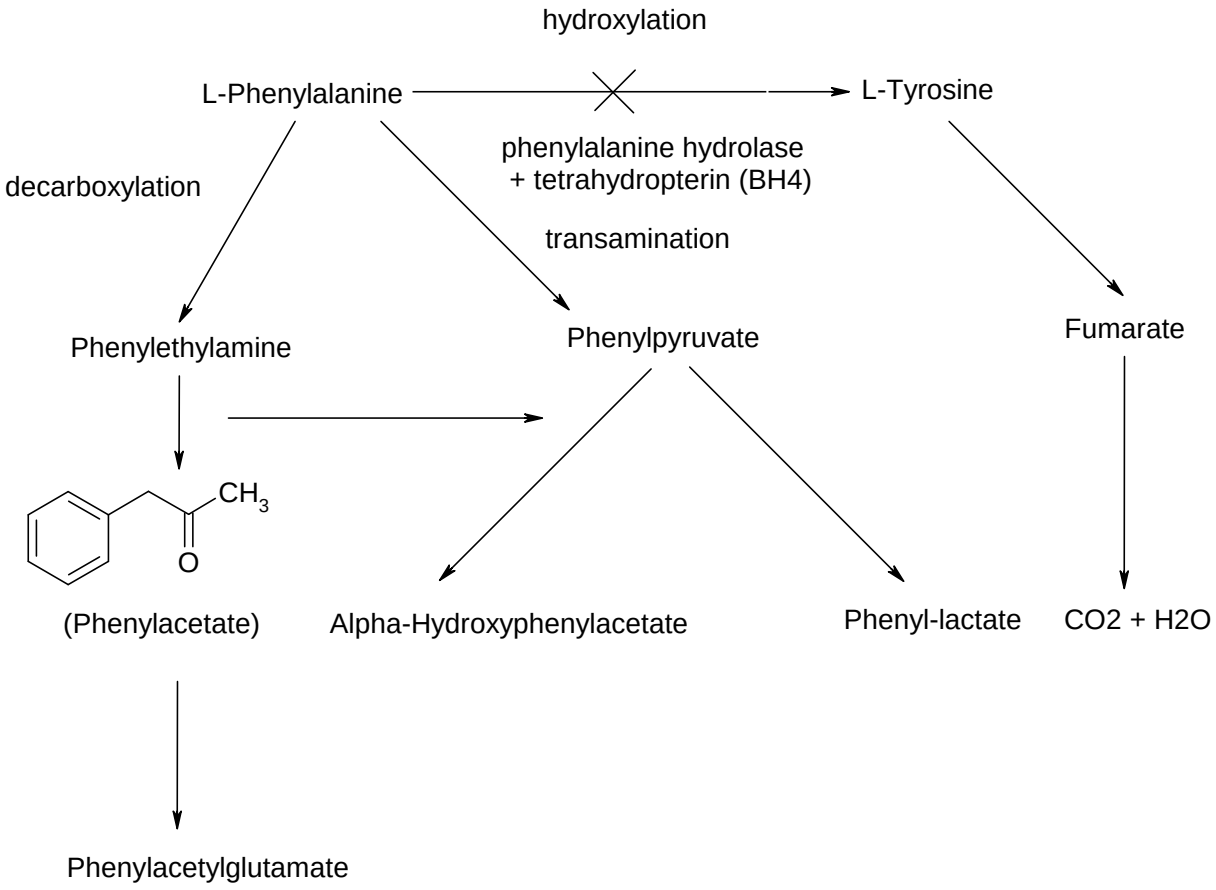


Figure 6: Biological pathway of phenylalanine in phenylketonuria [72]

Isovaleric acidemia

Isovaleric acidemia is characterised by an inherited leucine metabolism disorder which is followed by the accumulation of its derivates due to a deficiency of isovaleryl-CoA dehydrogenase, a mitochondrial enzyme responsible for processing amino acid leucine. (Figure 7) In case of its deficiency, leucine cannot be metabolised and its derivates (isovalerylglycine, isovalerylcarnitin and 3-hydroxyisovaleric acid) will accumulate in the blood and might also cause a serious damage to the central nervous system. The above mentioned metabolites are found in the urine samples as part of the newborn screening. Depending on the amount of these derivates, the patients with this disease have a specific odour, which is often described as cheesy, acrid or similar to sweaty feet. [73, 74]

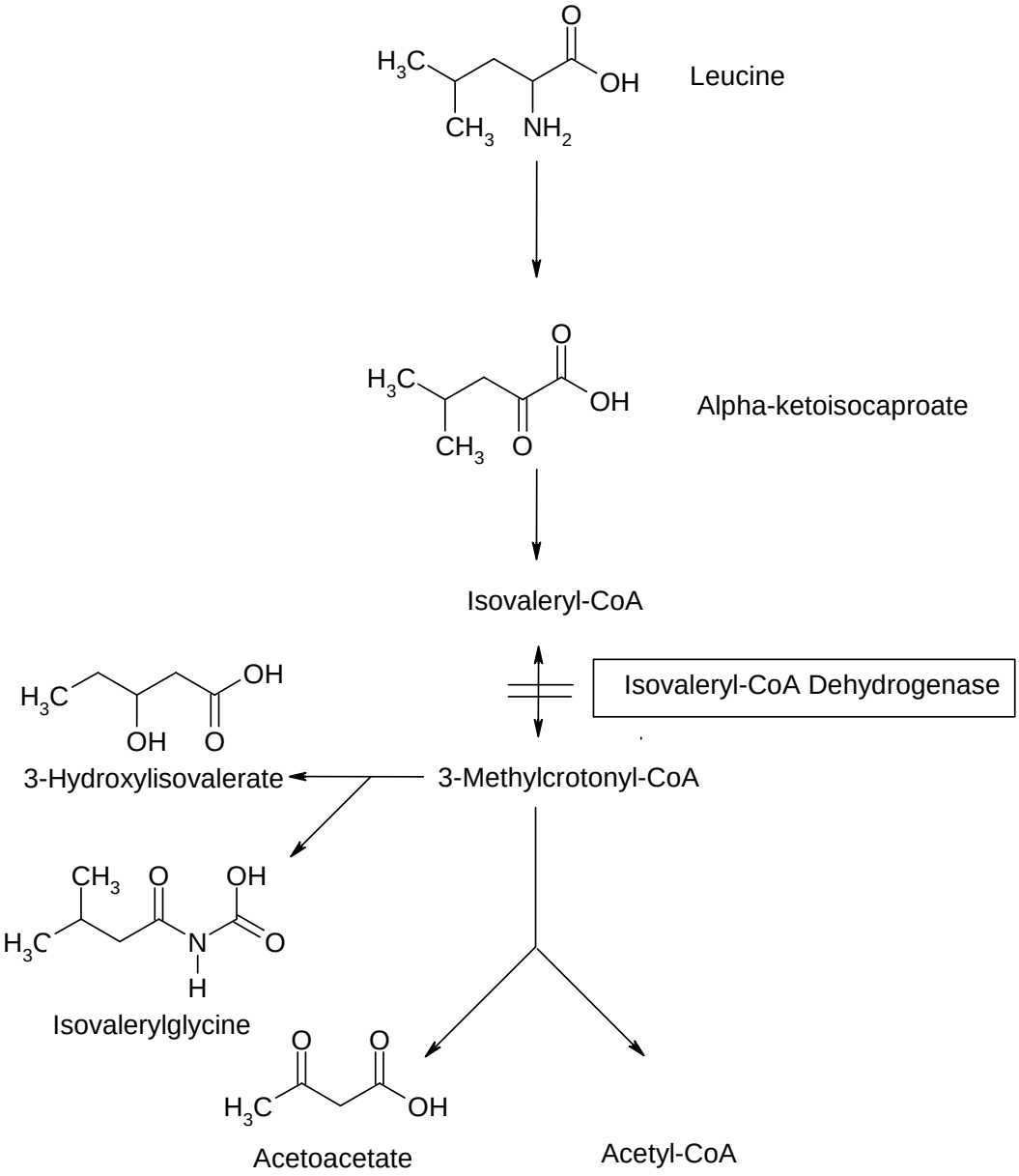


Figure 7: The catabolic pathway of leucine. Isovaleryl-CoA dehydrogenase catalyses the conversion of isovaleryl-CoA to 3-methyl-crotonyl-CoA. Enzyme deficiency results in the accumulation of isovaleryl-CoA derivates [75]

Maple syrup urine disease

This disease is another inherited defect in the metabolism of the branched-chain amino acids (leucine, isoleucine and valine), which therefore cannot be broken down through normal oxo-decarboxylation. The reason is a deficiency of an enzyme activity which catalyses the oxidative decarboxylation of 2-oxocarboxylic acids in the degradation of the branched-chain amino acids. (Figure 8) As a result, they accumulate in the blood and lead to serious damages to the central nervous system. Patients with this disease have elevated levels of the branched-chain amino acids and their products (ketoacids) in the blood, urine and ear wax, and are easily recognisable by their unique smell, similar to maple syrup or burnt sugar - hence the name, which is of great diagnostic importance, as those babies can be recognised soon after their birth. [76]

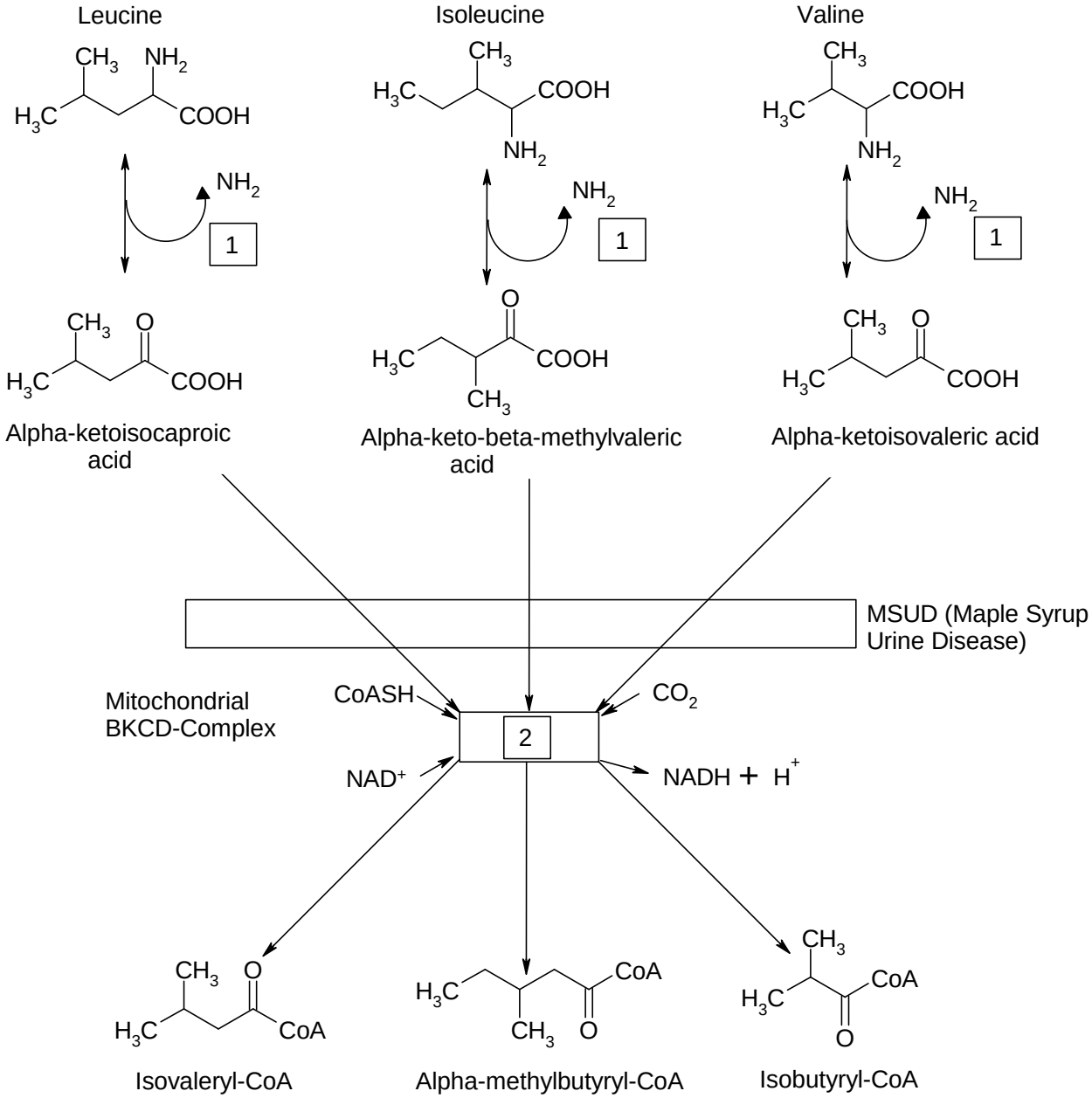


Figure 8: Metabolic pathway of branched-chained amino acids (leucine, isoleucine, valine). The transamination of leucine, isoleucine and valine is catalysed by a single branched-chain aminotransferase (reaction 1). The oxidative decarboxylation of branched-chain aminoacids is catalysed by the single mitochondrial branched-chain ketoacid dehydrogenase complex (BCKD, reaction 2). The metabolic block at the second reaction results in MSUD [77]

Hypermethioninemia

Due to a genetic deficiency in α/β-methionine adenosyltransferase, an enzyme responsible for transsulphuration, transmethylation and the biosynthesis of polyamines, methionine [78] accumulates in the blood causing neural demyelization and mental retardation. (Figure 9)

Patients do not show typical symptoms, but the urine and sweat of most patients with hypermethioninemia smell like a boiled cabbage.

The intensity of the smell depends on the amount of dimethylsulphide, a metabolite of methionine.

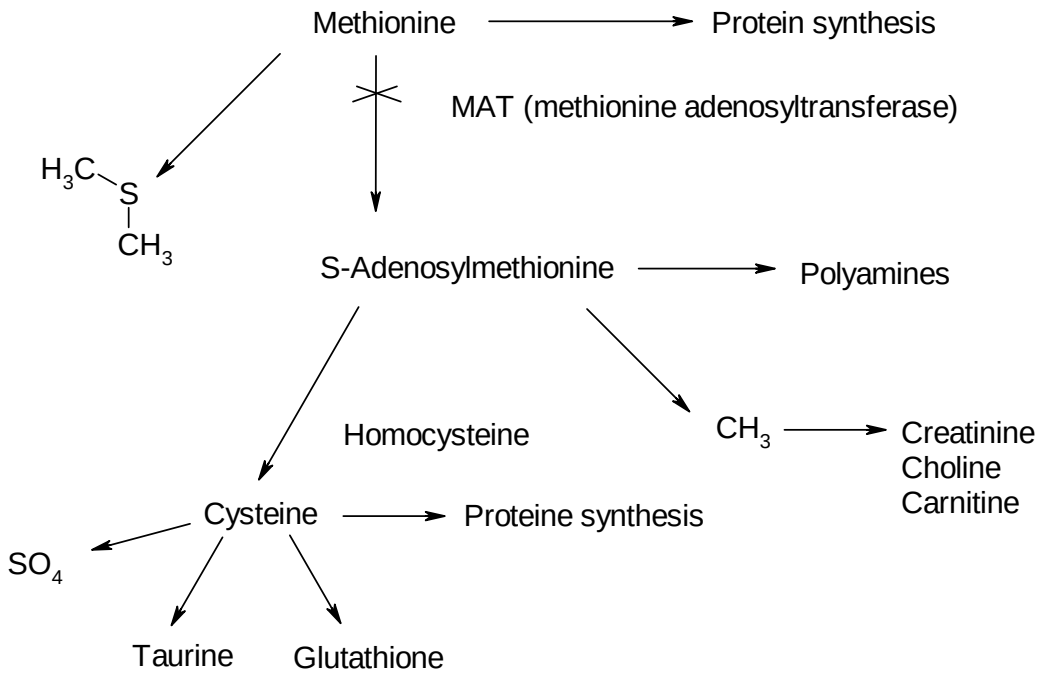


Figure 9: Hepatic transsulfuration pathway. Decreased MAT leads to the build-up of methionine and decreased downstream products [79]

Trimethylaminuria

In the past patients with this disease were associated with the fish malodour syndrome.

Trimethylaminuria is a rare and inherited metabolic disorder, in which individuals are not able to convert trimethylamine into a trimethylamine N-oxide, as a result of autosomal recessive mutation in the gene responsible for encoding flavin-containing monooxygenase enzyme 3. (Figure 10) In healthy individuals trimethylamine is converted into a non odorous compound trimethylamine N-oxide by flavin-containing monooxygenase enzyme 3 (FMO3) in the liver. [80, 81]

However, persons with trimethylaminuria have a reduced capability of oxidising trimethylamine into trimethylamine N-oxide which leaves trimethylamine unmetabolised in the body. This is excreted in urine, sweat, breath, saliva and reproductive fluids and has a strong rotten-fish like smell. [82]

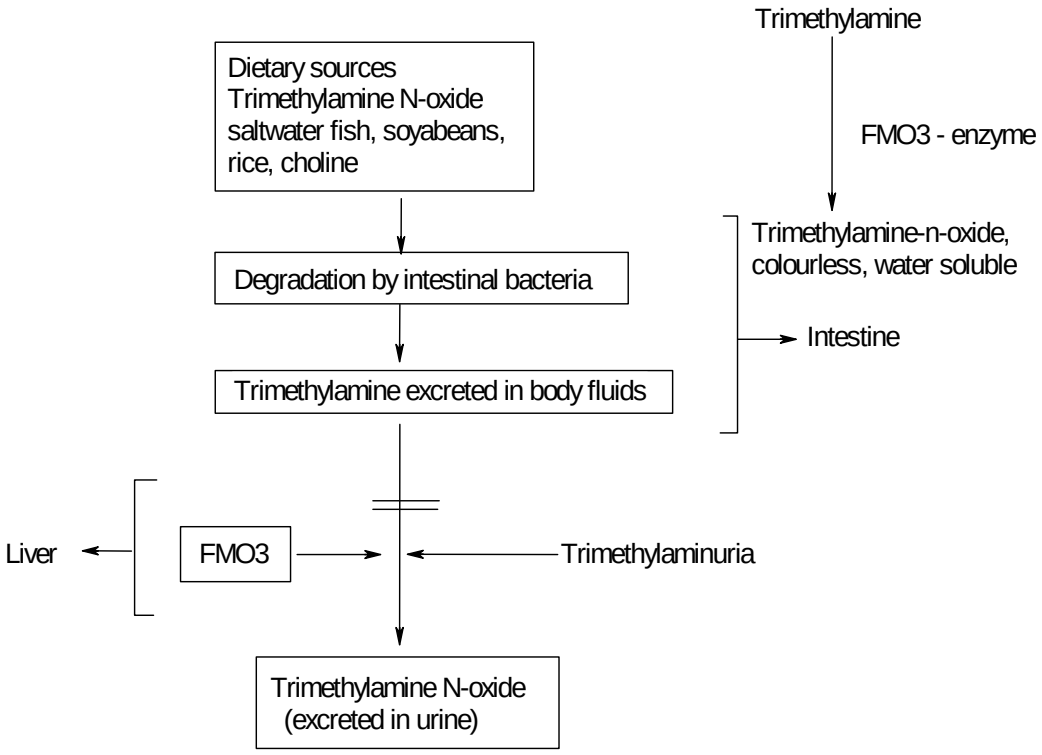


Figure 10: Metabolic pathway of trimethylamine.
FMO3 = flavin-containing monooxygenase [83]

Methionine malabsorption syndrome

Malabsorption of methionine in patients, also called Smith-Strang disease, is characterised by a conversion of part of unabsorbed methionine to α-hydroxybutyric acid by intestinal bacteria. The urine has an odour similar to that of dried celery, yeast or malt, or an oast house (a building for drying hops). Moreover, the patients have white hair and mental retardation. [84]

Cystinuria

Being an inherited, metabolic disorder, cystinuria is characterized by the accumulation of cystine crystals in the kidneys, urethra and bladder. Cystine cannot be properly re-absorbed into the bloodstream during the filtering process in the kidneys. Normally, this excess cystine is excreted in the urine, but in some cases the cystine cannot stay dissolved and forms crystals. As a sulphur-containing amino acid, the urine may have a characteristic "rotten egg" odour, which is attributed to the accumulation of putrescine, pyrrolidine and cadaverine. [85, 86]

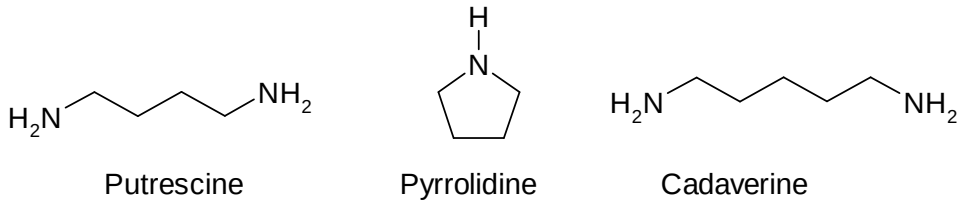


Figure 11: Putrescine, cadaverine and pyrrolidine

Tyrosinaemia

Tyrosinaemia is an inherited metabolic disease, in which the body cannot effectively break down the amino acid tyrosine, found in most animal and plant proteins. There are three types of tyrosinemia, each with distinctive symptoms and caused by the deficiency of a different enzyme. The most severe form is tyrosinaemia type 1, characterised by mutations in the gene encoding the enzyme fumarylacetoacetate hydrolase which catalyzes the final step in the degradation of tyrosine-fumarylacetoacetate to fumarate, acetoacetate and succinate. In patients with this disease, tyrosine, p-hydroxyphenylpyruvic acid and fumarylacetoacetate accumulate in the body. Those patients are recognisable by an odour similar to that of cabbage or rancid butter, which is here attributed directly to p-hydroxyphenylpyruvic acid. [85, 87]

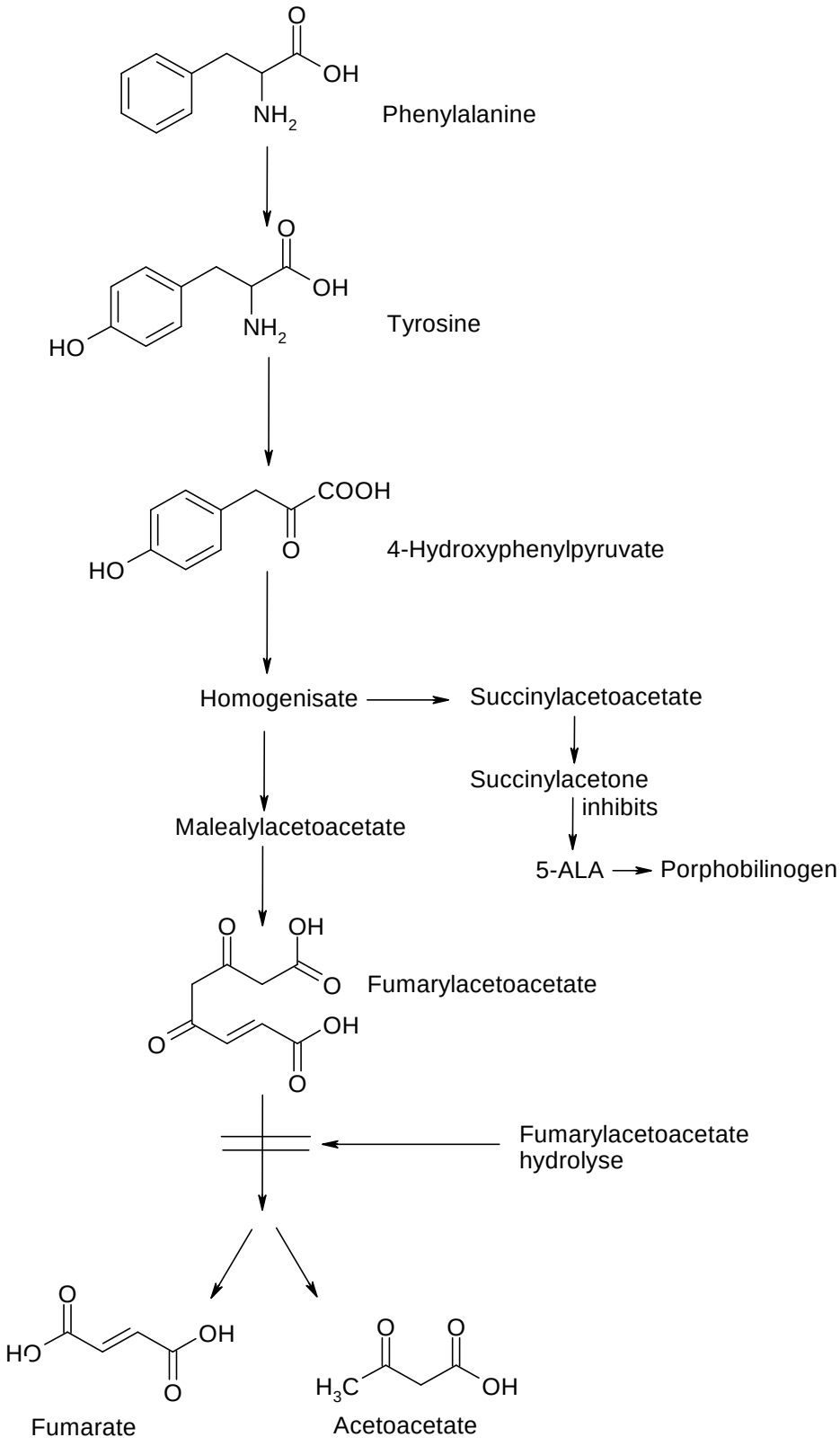


Figure 12: Metabolic pathway in tyrosinemia type 1. The defect in this disease and the metabolic block caused by a deficiency in fumarylacetoacetate hydrolyse is indicated. [87]

Diabetes Mellitus

Glucose is the main source of energy for our body and is used by cells for growth and energy. Insulin is a hormone made by the pancreas which helps glucose to enter our cells. In individuals with diabetes mellitus 1, no insulin is produced due to the destruction of the pancreatic beta cells. The blood sugar can reach extremely high levels due to the lack of insulin. This complication is called diabetic ketoacidosis presenting an emergency situation in most cases. The body loses its primary source of fuel and is forced to use fatty acids, which create ketone compounds (acetoacetate, 3-hydroxybutyrate and acetone). (Figure 13) These compounds are responsible for blood acidity, which later causes ketoacidosis of the blood. When excreted in the urine and breath of diabetics, they have a fruity smell which is attributed to acetone. [88]

At the same time, glucose from the food is excreted in the urine causing dehydratation. Other clinical signs of diabetic ketoacidosis are: vomiting, nausea, tachycardia, hypotension and Kussmaul respirations. [89]

Another type of diabetes mellitus, seen in adults and known as diabetes mellitus 2, is characterised by insulin resistance and abnormal insulin secretion. Its main clinical manifestations are: polyuria, polidipsia and weight loss.

The main complication is a hyperglycemic, hyperosmolar state, characterised by high blood sugars, increases in osmolarity and a high risk of further complications, such as coma and death. Notably absent are symptoms of nausea, vomiting and Kussmaul breathing. Unlike diabetic ketoacidosis ketone bodies are often not detectable. [89]

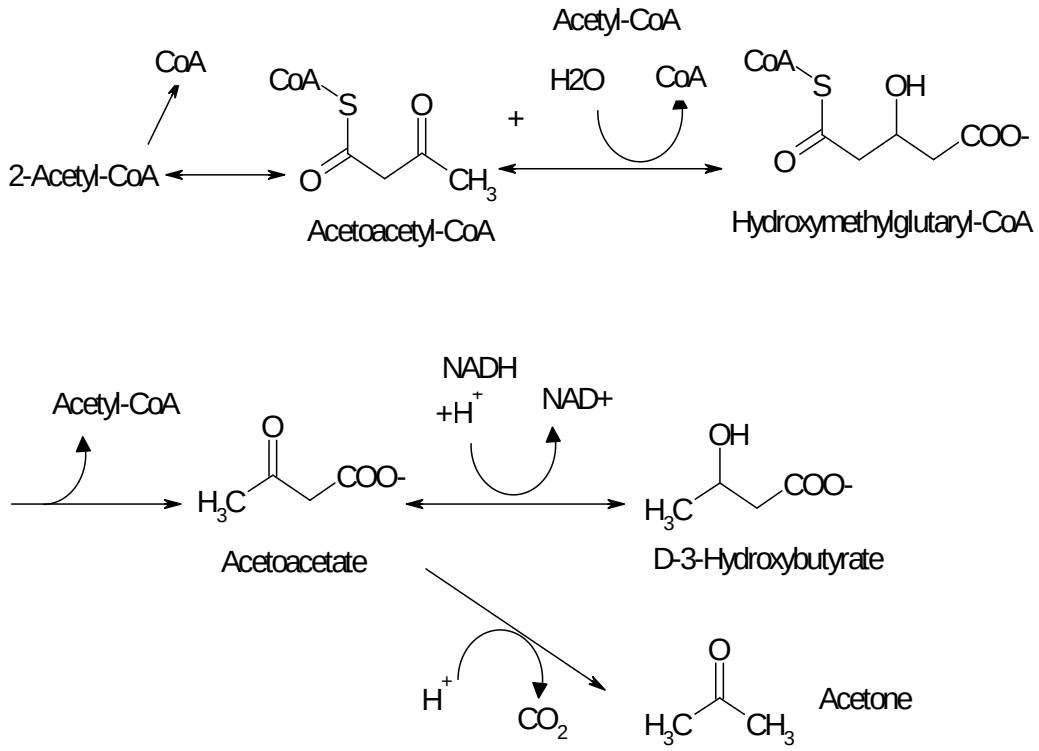


Figure 13: Metabolic pathway in diabetes mellitus [88]

Novak et al. [90] identified VOCs of patients with diabetes mellitus type 1, who also showed a significant hyperglycemia before their breath samples were collected and analyzed with GC-MS.

These results showed that the exhaled methyl nitrate was statistically strongly correlated to that of blood glucose. The authors also suggest that oxidative processes play a major role in the biochemical production of that gas. A small fraction of oxygen is converted in the mitochondria to superoxide ion ($O2^-$), which can damage cells and tissue. This is prevented by antioxidant mechanisms including the activation of superoxide dismutase (SOD), which converts

superoxide to the less reactive oxygen and hydrogen peroxide by adding protons. The superoxide can also react rapidly with nitric oxide (NO) forming a nitrate molecule. In case of hyperglycemia, an accelerated metabolic flux through the mitochondria leads to the formation of superoxide, probably linking blood glucose levels with systematic oxidations. This chain of reactions might be accelerated by a PH shift towards acidosis in the case of hyperketonemia.
Therefore, the exhaled methyl nitrate might be used as a future screening tool for detecting this type of diabetes in children. (Figure 14)

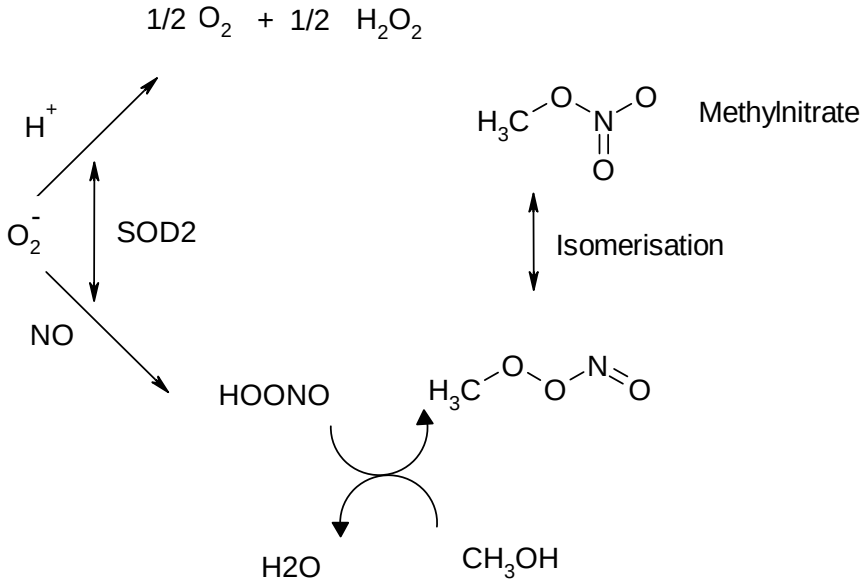


Figure 14: Schematic representation of methyl nitrate formation in vivo. In vivo, a small but relatively constant fraction of superoxide ion ($O2^-$) is derived from its interaction with superoxide dismutase (SOD) and reacts with nitric oxide (NO) [90]

Uremia/kidney failure

Uremia is a type of kidney failure which is characterized by the accumulation of excessive nitrogenous waste products (urea) in the blood, due to a failure to filter the blood correctly. Since urea is broken down to ammonia and trimethylamine, patients exhale breath with an ammonia or urine-like odour. [91]

5. INFECTIOUS DISEASES

The microbial species in infected persons are known to produce various VOCs, mostly due to the interaction between the host organic media or biological fluids with microbial toxins. Most of these VOCs include aldehydes, esters, alkanes or alcohols and are normally released in breath or faeces. The clinicians were familiar with the smell of different infectious diseases in the past. Patients with scrofula were, for example, known to emit a fermentative odour similar to stale beer. Unpleasant odours are often produced from certain respiratory tract diseases including bronchiectasis, lung abscesses, and ozaena. Patients with typhoid fever produce a smell comparable to freshly baked brown bread, whereas individuals with diphtheria have a sweet or putrid odour in their breath.

However, in most cases the odour of the afflicted patients is perceived with bacterial infections.

Bacterial infections

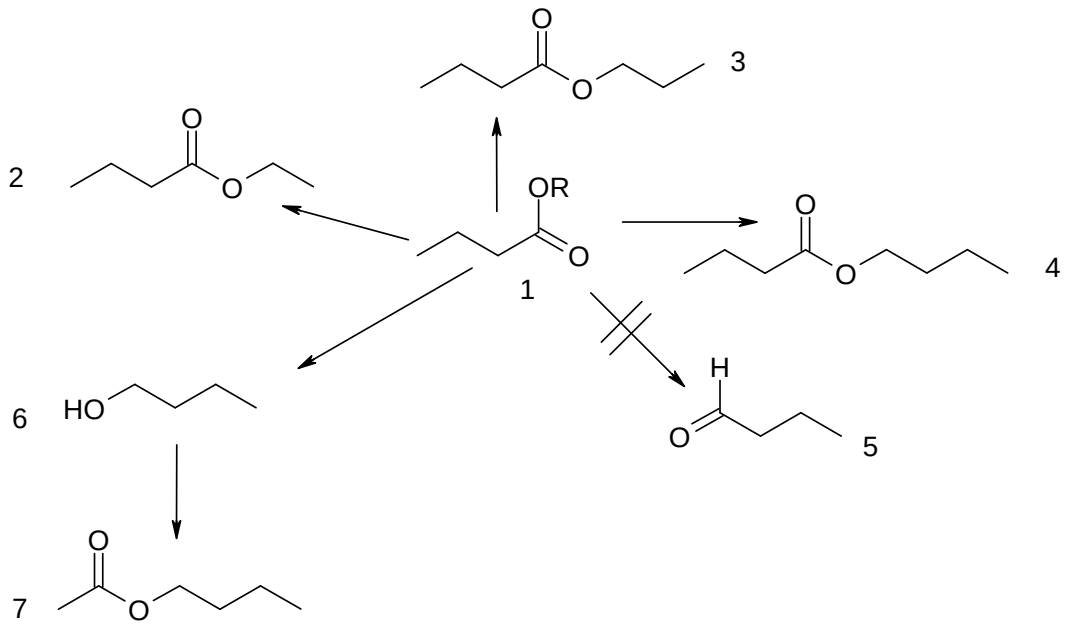
The abnormal smell of the faeces is often associated with infectious gastrointestinal diseases. However, little has been known about the abnormal smell of the faeces.

Garner et al. [11] investigated the VOCs in the faeces of healthy volunteers compared to patients infected with *Clostridum difficile* and *Campylobacteri jejuni*, as well as patients with ulcerative colitis. In their study a total of 297 VOCs from faeces were identified by GM/MS in both healthy and sick volunteers. A total of 149 VOCs were identified in patients with *Clostridium difficile*, 183 in patients with *Campylobacteri jejuni* and 145 VOCs in patients with ulcerative colitis.

The large number of VOCs in asymptomatic patients is derived from intestinal microbiological metabolism of foodstuff and is not associated with any gastrointestinal diseases.

The results suggest that microbial mediated reduction of fatty acids occur (Figure15) with high levels of butanoic acid as a VOC, present in all infected

patients with *Campylobacteri jejuni* and ulcerative colitis, but not in patients infected with *Clostridium difficile*.



1.R= OH
2.R= ethyl
3.R= propyl
4.R= butyl
5.Butanal
6.1-Butanol
7.Ethanoic acid butyl ester

Figure 15: Products found from the conversion of butanoic acid after incubation with fresh stool from an asymptomatic donor [11]

Contrary to this finding, butanol was ubiquitous in *Clostridium difficile* faeces samples. *Clostridium difficile* also produces ethanol and isopropanol but less butanoate, which explains these findings.

On the other hand, 1-octen-3-ol was not identified from faeces samples containing *Clostridium difficile*, but was present in high levels in patients with *Campylobacteri jejuni*. Low levels of alkanes were identified from faeces samples of patients with both *Clostridium difficile* and *Campylobacteri jejuni*, but in high concentrations in faeces samples of patients with ulcerative colitis. However, patients with ulcerative colitis had low levels of nitrogen-containing compounds.

All asymptomatic patients (healthy volunteers) had elevated levels of dimethylsulphide, trimethylsulphide, carbon disulphide and methanethiol.

The abscence of 1-butoxy-2-propanol was also specific of asymptomatic patients, but found in high concentrations in stools of *Campylobacteri jejuni* patients.

The abscence of 2-(3)-methylfuran was specific of the patients with *Clostridium difficile*, *Campylobacteri jejuni* and ulcerative colitis.

High levels of 1-butoxy-2-propanol were found in stool samples of *Campylobacteri jejuni.*

Likewise, the abscence of 2-(2-ethoxyethoxy)-ethanol, toluene and hexanoic acid is characteristic of faeces samples with C. difficile.

In another publication dealing with the VOCs of cholera patients in Bangladesh [92], it has been reported that fewer VOCs were detected from cholera patients and contrary to Garners previous publication high levels of dimethylsulphide were detected from cholera patients which were absent in asymptomatic volunteers.

However, high levels of p-menth-1-en-8-ol were also found, which might be a future biomarker in the early detection of cholera.

Garner et al. [93] also investigated the VOCs of infants, and those released in infants with necrotising enterocolitis. The study suggests that infants with necrotising colitis had fewer esters than their healthy counterparts.

2-ethylhexyl acetic ester, decanoic acid ethyl ester, dodecanoic acid ethyl ester, and hexadecanoic acid ethyl ester disappear from the faeces 4 days later, thus making them a possible biomarker for this disease in the future.

Being a leading cause of death from all infectious diseases, active pulmonary tuberculosis has to be diagnosed with new and more accurate screening tests. Phillips et al. [94] assessed the information on the VOCs released from both

Mycobacterium cultures, as well as from patients suffering from active pulmonary tuberculosis (TBC). TBC may alter the VOCs in breath, but it is also important to notice, that patients with TBC also suffer from oxidative stress. The aim of this study was to identify the VOCs characteristic of *Mycobacterium tuberculosis*, as well as to determine whether TBC in vitro and patients with active TBC produce distinctive VOCs. The VOCs in this study were also identified by GC/MS.

A total of 130 different VOCs were isolated in vitro, predominately derivates of benzene, naphthalene and alkanes.

The 10 most abundant VOCs derived from *Mycobacterium* culture were:

1. 1- Methyl-naphthalene
2. 3-Heptanone
3. Methylcyclododecane
4. 2, 2, 4, 6, 6-Pentamethyl-heptane
5. (1-Methylethyl)-1-methyl-4-benzene
6. 1,4-Dimethyl-cyclohexane
7. 3,5-Dimethylamphetamine
8. 3-Methyl-Butanal
9. 2-Hexene
10. Trans-anti-1-methyl-decahydronaphthalene

The 10 most abundant VOCs derived from the breath of patients, who had a positive sputum test against *Mycobacterium* tuberculosis were:

1. Ethyl-benzene
2. Methyl-benzene
3. Propyl-benzene
4. 3-Methyl-heptane
5. 2-Methoxy-2-methyl-propane
6. 1-Octene
7. Cyclohexane
8. 2-Butyl-1-octanol
9. 1-Methyl-naphthalene
10. 1,4 -Dichloro-benzene

These results show, that the cultured *Mycobacterium* species and patients with an acute pulmonary tuberculosis release distinctive VOCs.

In another publication Phillips et al. [95] suggest that the VOCs released in the breath of patients with *Mycobacterium tuberculosis* are derived from the infected host (oxidative stress) and are produced from TBC.

The main VOCs of TBC are cyclohexane and benzene derivates, similar to those derived from cultured *Mycobacterium* species. The VOCs released due to an oxidative stress include alkane and alkane derivates.

These VOCs identifed from high-risk patients with acute pulmonary tuberculosis have 85 % accuracy.

Filipiak et al. [96] studied the VOCs of patients with ventilator-associated pneumonia, infected with *Staphylococcus aureus* and *Pseudomonas aeruginosa* by using GC-MS. Since pneumonia is one of most common deaths of infectious diseases, this group studied the release of the VOCs in patients with this pneumonia with two most common nosocomial bacteria (*S. aureus* and *P. aeruginosa*) with the objective of developing a non-invasive test for diagnosing this bacterial pneumonia.

A total of 32 VOCs were released by *S. aureus* and 37 VOCs were released by *P. aeruginosa*, many of which were aldehydes, (propanal, 3-methyl-2-butanal, benzaldehyde, acetaldehyde, 3-methyl butanal, 2-methyl propanal) and acids (isovaleric acid and acetic acid). A total of 37 VOCs were released from *P. aeruginosa*.

Both bacteria produce alkanes. The short-chained (4-C-atoms) were released by *S. aureus* and long-chained alkanes by *P. aeruginosa*.

Ketones produced by *S. aureus* include hydroxybutanone and hydroxyacetone. Worth mentioning is that, entirely different ketones were released by *P. aeruginosa*, comprising 2-butanone, 2-pentanone, methyl isobutyl ketone, 2-heptanone, 4-heptanone, 3-octanone and 2-nonanone.

It is important to stress, that *S. aureus* produces acids such as isovaleric acid and acetic acid (Figure 16), whereas no acids were produced by *P. aeruginosa*.

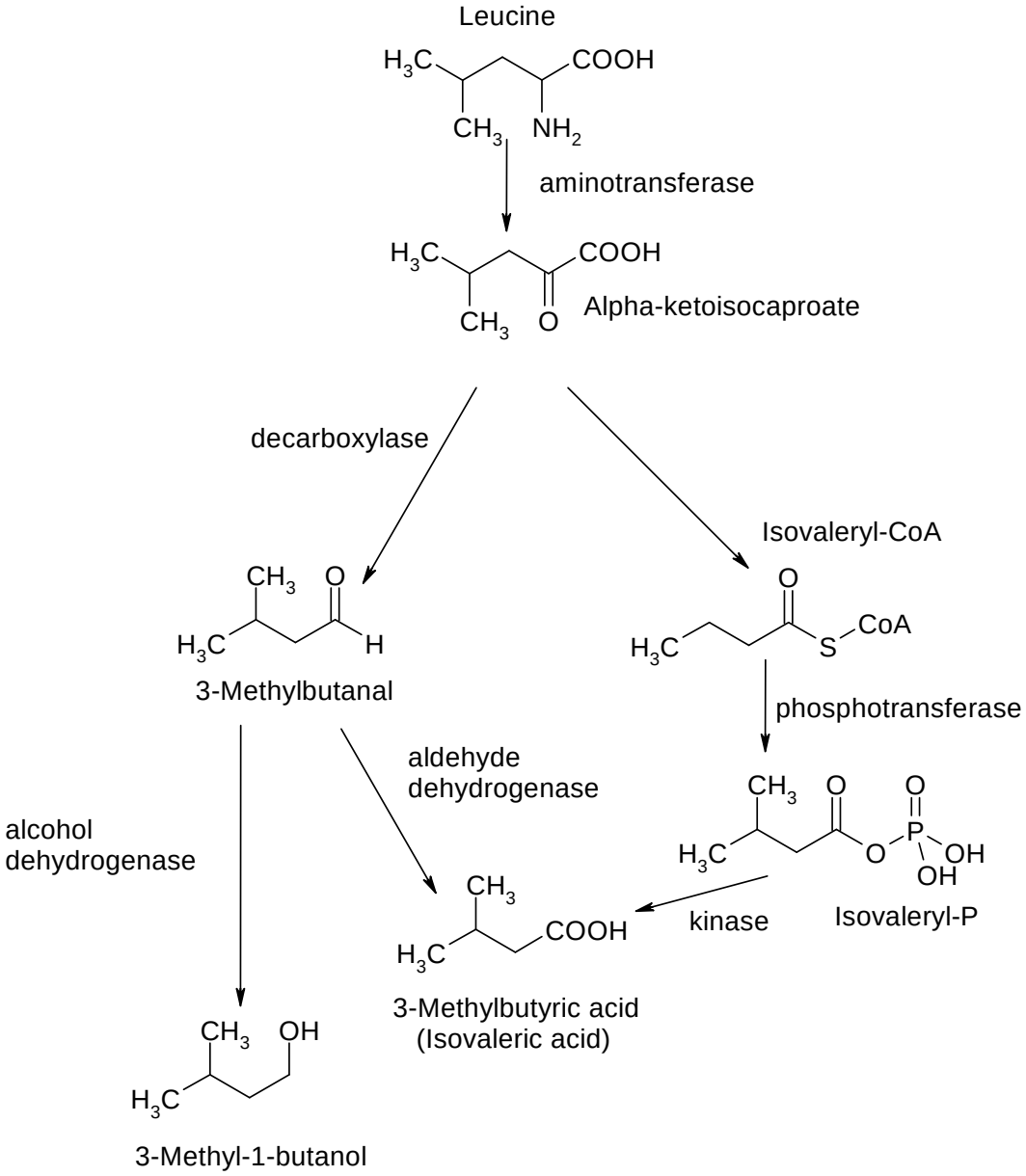


Figure 16: Catabolism of leucine leads to the formation of 3-methylbutanal, 3-methyl-1-butanol and 3-methylbutyric acid (isovaleric acid), which were found to be significantly released by *S.* aureus [96]

The metabolic origin of VOCs produced by both bacteria is not completely elucidated, but it has already been described, that production of branched-chain aldehydes results from the catabolism of amino acid. (Figure 16)

Since all compounds found in this study were identified in vitro, it is presumable that amino acid degradation and not synthesis of fatty acids from alkanes serves as the underlying pattern of VOCs released by *S. aureus*, especially since the culture medium consisted mainly of amino acids, peptides and glucose.

The catabolism of pyruvate (Figure 17) plays an important role in case of *S. aureus* since the products of this metabolic pathway were found in the headspace of this bacterium including ethanol, acetaldehyde, acetic acid and acetoin.

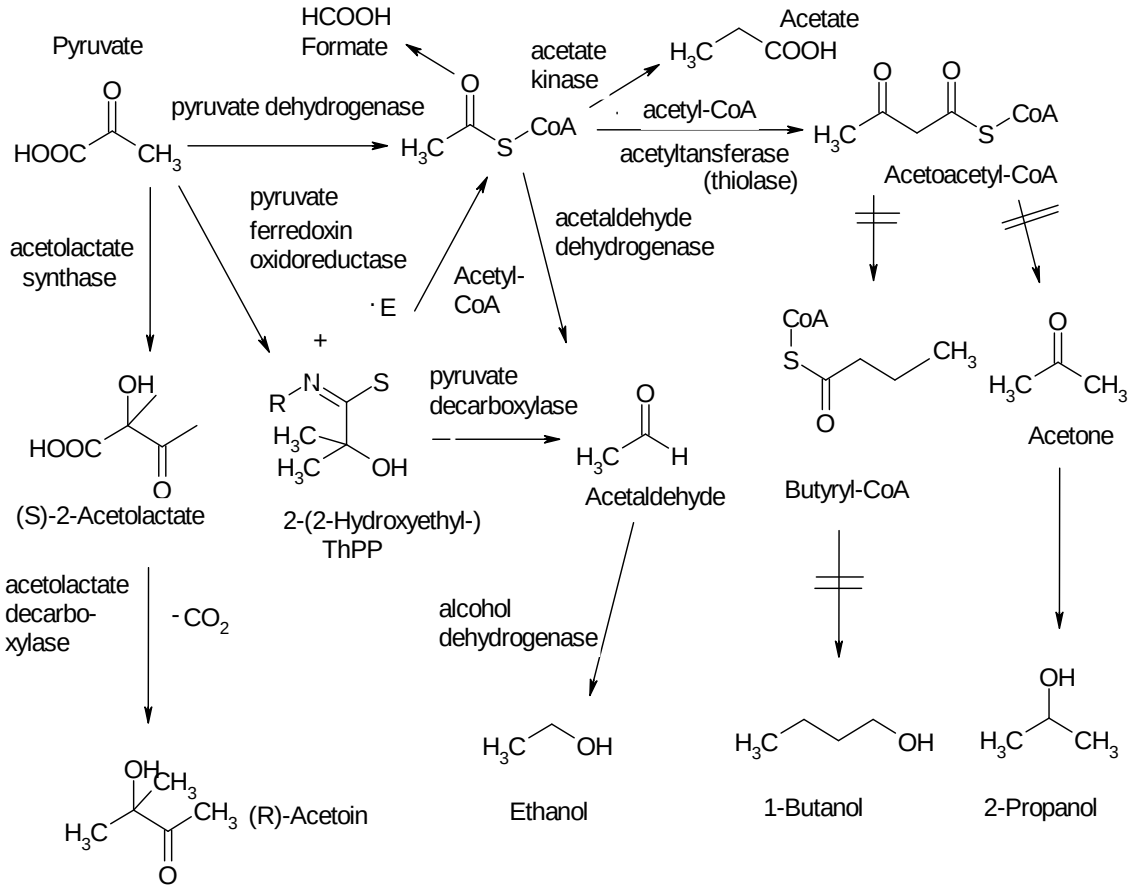


Figure 17: Simplified scheme of pyruvate metabolism via glycolytic fermentations and lactate converting fermentations, modified after Michel et al [96]

Pathways which lead to the production of VOCs significantly released by *S. aureus* in this study are presented, including acetoin (3-hydroxy-2-butanone), acetaldehyde, ethanol, 1-butanol, acetone and 2-propanol. In case of *P. aureginosa* the metabolism of amino acids, rather than glycolysis of carboxyhydrates yields pyruvate as starting material.

Interestingly, no acids and aldehydes were released by *P. aeruginosa*.

Pseudomonads are also known as organisms with strictly respiratory metabolism mainly with oxygen and in some species nitrate as terminal electron acceptor; hence the release of alcohols and acids from these microorganisms is not expected.

Both bacteria also produce sulphur-containing compounds, such as dimethylsulphide, dimethyldisulphide and dimethyltrisulphide which originate from auto-oxidation of methanethiol that can be produced via metabolism of the sulphur-containing amino acids, e.g. via demethiolation, transamination or recombination.

The early and strong release of nitrogen-containing compounds, such as pyrrole, 1-vinylaziridine and 3-methylpyrrole by *P. aeruginosa* could be a biomarker for the early detection of this bacterium.

Likewise, the results with α-unsaturated hydrocarbons, such as 1-undecene and 2-nonenone, indicate that these VOCs might also be typical biomarkers for *P. aeruginosa*, since they are not found in the exhaled breath samples, but are significantly released after inoculation with this gram negative bacterium. In the same way, acetoin and acetol meet all requirements for a perfect biomarker of *S. aureus*.

These results suggest that different bacteria might have a different odour "fingerprint“ in infected patients, which might be helpful in the early detection of various infectious diseases.

Viral and fungal infections

In most cases a specific odour of viral infections is not perceived. Patients with smallpox, who after at least 2 weeks of inoculation have a pus-filled rash over the

entire body, might have a sweetish or pungent odour originating from the infected lesions. [97]

Yellow fever is a viral disease caused by the yellow fever virus, which is transmitted to humans by female mosquitoes. Patients with yellow fever often have a body odour that smells like a butcher’s shop. [98]

The fungal infections are quite rare in healthy patients and occur frequently as vaginal infections. The fungi in vaginal secret are known to have a fishy odour due to the presence of diamines. [99]

6. OTHER DISEASES

Asthma bronchiale

Current approach to diagnosing asthma bronchiale is almost entirely based on clinical observations and lung function tests.

These tests are often not specific of only one lung disease, which raised the question of seeking new and effective screening methods for asthma bronchiale. One of them might include the analysis of VOCs from exhaled breath of persons, who are believed to suffer from asthma bronchiale.

Paredi et al. [100] studied the exhaled ethane concentration and NO concentration of 26 patients, affected with asthma bronchiale, most of whom were on a steroid therapy, and 16 healthy controls, who were non-smokers. Ethane is known to be a marker of lipid peroxidation, which was also present in high levels in patients with lung cancer. On the other hand, NO is known to be an inflammation marker.

The results of this group suggest that both ethane and NO concentrations were elevated in patients with asthma bronchiale, compared to healthy controls. Moreover, patients with severe asthma bronchiale had higher concentrations of ethane and NO compared to patients with mild asthma bronchiale and healthy controls.

Those with severe asthma receiving a steroid therapy had significantly lower levels of ethane and NO, compared to untreated patients, which suggests that oxidative stress and lipid peroxidation are increased in the airways of asthmatic patients. This is probably due to an activation of neutrophils, macrophages and eosinophils, which may lead to oxidation of nucleic acids, proteins, and membrane lipids in the airway of asthmatic patients. The high concentrations of NO are explained by an increased inflammation in the airway. According to this study, both ethane and NO might be used as future biomarkers of asthma bronchiale.

Since the impact of oxidative stress on asthma bronchiale is very important, Montuschi et al. [101] studied the concentration of 8-isoprostane in exhaled breath condensate of asthmatic patients.

Belonging to the F2-isoprostane class and being another biomarker of oxidative stress, 8-isoprostanes are free radical-catalyzed products of arachidonic acid,

formed in situ in cell-membrane phospholipids, from which they are cleaved by phospholipase A. (Figure 18) [102]

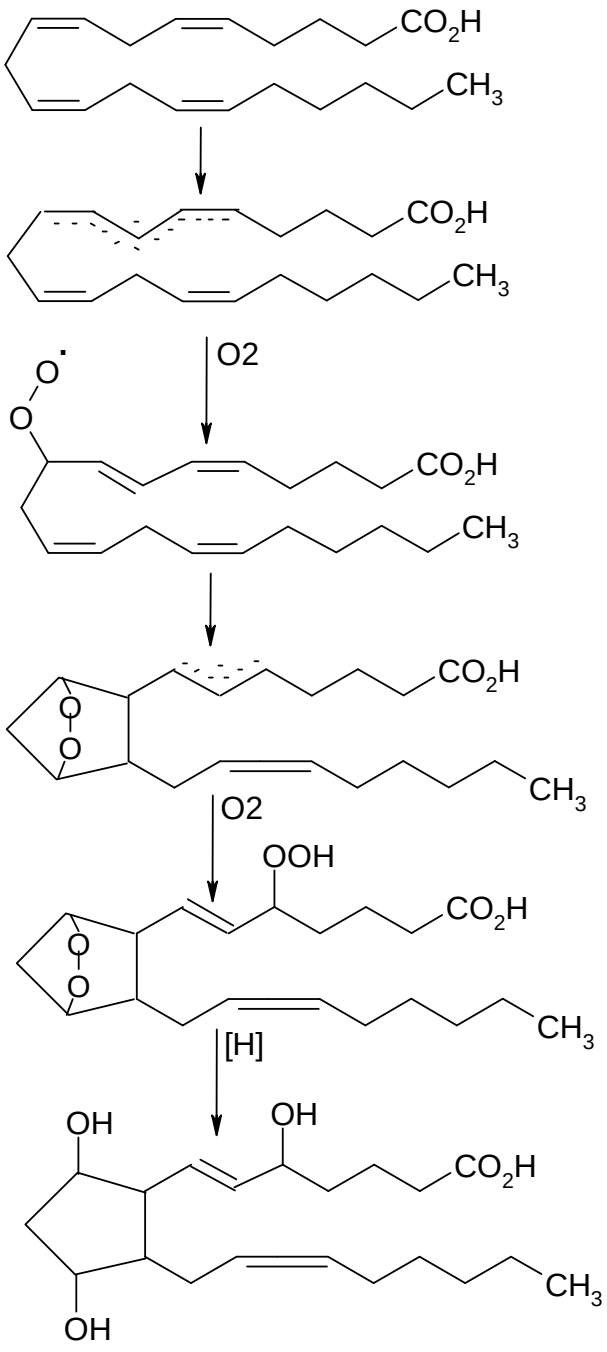


Figure 18: Mechanism of formation of the F2-isoprostanes. This pathway leads to the formation of four regioisomers. For simplicity, stereochemical orientation and other 3 isomers, which are not contained in the exhaled breath as VOCs, are not indicated [102]

High levels of 8-isoprostane have already been found in patients with hepatorenal diseases, paracetamol intoxications and recently in the urine of COPD-patients. [103]

Four groups of participants were included: 10 healthy persons, 12 patients with mild asthma, 17 patients with moderate asthma and 15 patients with severe asthma. The results suggest that 8-isoprostane concentrations were detectable in all groups, being however, significantly higher in patients with asthma. In patients with mild asthma the increased concentrations were doubled and in patients with severe asthma increased by 3-fold.

Similarly, the exhaled concentrations of NO were also increased in patients with mild asthma, but not in those with moderate or severe asthma. The exhaled concentrations of CO were also increased in patients with mild and severe asthma, but not in those with moderate asthma. These findings regarding the concentrations of CO are counterintuitive and the authors do not give us a possible explanation why the concentrations of CO in patients with moderate asthma are decreased. One of the possible explanations might be a result of a corticosteroid treatment, which significantly decreases the concentration of CO. [104, 105] This might contradict the results of increased concentrations of CO in patients with severe asthma, but knowing that corticosteroids are less efficient in controlling higher levels of oxidative stress in advanced stages, such results are expected. The concentrations of CO are also increased in mild asthma, since no corticosteroids are administered in this stage of the disease.

8-isoprostane might be a future biomarker for screening this disease, as its concentration is not influenced by a corticosteroid therapy.

Rumchew et al. [106] investigated a domestic exposure of children to VOCs in Australia. Their results suggest, that a domestic exposure to benzene, ethylbenzene and toluene may increase the risk of childhood asthma thus indicating that some of the VOCs released by asthmatic patients are also air pollutants and their increased concentration in exhaled breath is not only related to asthma.

Furthermore, it is not always possible to determine the level of pollution and concentration of these VOCs in our environment.

Dallinga et al. [107] studied the VOCs in exhaled breath of children, as children are active non-smokers and are also less exposed to air-pollution. The aim of their study was to identify a specific pattern of VOCs in asthmatic children. A total of 120 children were recruited, 63 of whom were diagnosed for asthma and 57 of whom were healthy controls.

They identified 8 VOCs by GC/MS. These were increased in asthmatic children with a sensitivity of 89 % and a specificity of 91 %.

1.) (Branched) hydrocarbons
2.) Carbon disulphide
3.) 1-Penten-2-one
4.) Butanoic acid
5.) 3-(1-Methylethyl)-benzene
6.) Unsaturated hydrocarbons
7.) Benzoic acid
8.) p-Xylene

Dragonieri et al. [108] used an electronic nose in distinguishing patients with asthma from healthy controls. Ten young patients with asthma, ten young healthy controls, ten older patients with severe asthma and ten older healthy controls were recruited. The results suggest that electronic noses could distinguish smellprints of ten young asthmatic patients and separate them fully from ten young healthy controls with 100% accuracy.

Similarly, electronic nose could distinguish smellprints of older asthmatic patients from older healthy controls with 90% accuracy.

However, patients with mild and severe asthma could be less well discriminated (65 % accuracy).

These data suggest a possible use of electronic noses in diagnosing asthma, as well.

COPD

Chronic obstructive pulmonary disease (COPD) is an inflammatory disease, characterised by an oxidative stress and the formation of VOCs that are released into the environment via lungs.

Basanta et al. [109] identified a total of 487 VOCs from 71 human subjects, 39 of whom were COPD-patients and 32 healthy controls.

The main VOCs identified by GC/MS with a sensitivity of 85 % were:

1. Undecanal
2. Hexanal
3. Dodecanal
4. Decanal
5. Nonanal
6. Pentadecanal
7. Oxirane
8. Cyclohexanol
9. Butanoic acid
10. Pentanoic acid
11. 2-Pentyl-furane

According to the authors, the origin of those VOCs is unknown.

However, the authors suggest that aldehydes (the first 6 VOCs) might be elevated in patients with COPD due to the metabolic upregulation in the mucosa of COPD patients, which removes aldehydes from the air.

Their findings also indicate that it is possible to use this method in diagnosing COPD in the future and also to distinguish different phenotypes of COPD according to the level of the above mentioned VOCs. Since COPD and asthma can exhibit overlapping clinical symptoms, it is very difficult to differentiate between these two diseases before treatment is applied.

Fens et al. [110] used electronic noses in distinguishing COPD patients from asthma bronchiale and from healthy controls. The results suggest that electronic noses

could distinguish patients with COPD from asthmatic patients, as well as healthy controls from both diseases, which further strengthens the hypothesis, proposed by Dragonieri et al. [108], that electronic noses may become very useful diagnostic tools in diagnostic COPD and asthma bronchiale in the future.

Cardiovascular diseases

Since cardiovascular diseases are a leading cause of death in the industrialised world, there is a rush for new diagnostic methods, as early detection of those diseases, accompanied by a medical treatment might prevent the increasing mortality.
However, very few studies have been made as far as the study of VOCs in such patients is concerned.

Phillips et al. [111] published their work on the VOCs from exhaled breath of patients with unstable angina pectoris and from healthy controls. Breath samples are analysed from 30 patients with unstable angina pectoris, whose diagnosis was confirmed by coronary angiography, as well as 38 breath samples from patients with no known history of heart disease.
The following VOCs were found in higher concentrations in patients with unstable angina pectoris:

1. 4-Methyl-octane
2. 4-Methyl-decane
3. Hexane
4. 5-Methyl-pentadecane
5. 7-Methyl-hexadecane,
6. 2-Methyl-propane
7. Pentane
8. 2-Methyl-butane

Since alkanes and methylated–alkanes are known to be biomarkers of oxidative stress, it is certain, that patients with unstable angina pectoris also suffer from an

oxidative stress. For that reason, future clinical trials are necessary to evaluate breath samples, in order to differentiate between cardiac and non-cardiac chest pain. The same VOCs might also be released in persons suffering from non-cardiac chest pain, since an oxidative stress is also linked to non-cardiac chest pain.

Cikach et al. [112] identified NO as a possible marker in cardiovascular diseases. According to their findings, the NO-concentration was significantly lower in patients with heart failure and pulmonal hypertension.

Schizophrenia

Schizophrenia is a severe mental disorder, affecting nearly 1 % of the world's entire population, characterised by hallucinations and cognitive defects. It is also believed, that specific genetic variants are associated with certain types of this disease.

In the mid 60-ies a peculiar smell was often perceived in psychiatric hospitals, linking the smell with trans-3-methyl-2-hexenoic acid in the sweat of patients with schizophrenia.

Years later, Gordon et al. [113] proved that the same acid was also present in the sweat of normal persons, indicating that there was no special relation between this acid and schizophrenia.

Phillips et al. [114] identified the VOCs from patients' breath. They recruited 51 patients with schizophrenia and 37 healthy controls. They found elevated levels of pentane and disulphide in the exhaled breath of the patients, thus indicating that patients with schizophrenia were subject to oxidative stress and lipid peroxidation. As a result, high levels of ethane were measured in the exhaled breath. However, the origin of carbon disulphide, which is a neurotoxin, was unclear. Two years later, the authors published another article, in which they described a VOC–pattern, which distinguishes patients from schizophrenia from healthy controls. [115]

This pattern includes: 2-methylbutane, trichlorofluoromethane, 2-pentanol, pentane, dichloroethane, trichloroethene, benzene, 1-chloro-2-methylbutane, 2,3,3-trimethylpentane, 2,2-dimethylbutane and tetrachloroethene with a sensitivity of 80 % and a specificity of 69%.

Since diagnosing schizophrenia is very difficult and based primarily on clinical observations, these results might be a significant step towards a non-invasive diagnosing schizophrenia.

7. CONCLUSION

With this paper we carried out a review of the study of VOCs released from the human body and their scientific evidence in creating a unique chemical signature or “smellprint”, which can be detected in certain diseases, thus emerging as a new field of scientific research of growing importance. With the help of modern instruments, VOCs can be detected and analysed in the form of “fingerprints”, which are disease-specific.

Most VOCs are released through the exhaled breath, which can be easily collected and analysed. The identification of these VOCs has led to important findings, suggesting that unique “VOC patterns” are characteristic of certain cancer types, especially of lung cancer. Data on identifying and quantifying the VOCs released from lung cancer cell-lines cultured in vitro and being similar to the VOCs released from the exhaled breath of patients with lung cancer, support the above mentioned findings. Based on the assumption, that VOCs specific of lung cancer are already known, screening of VOC- profiles may lead to the development of “pulmogram“ of lung cancer, since when recognised at a very early stage, lung cancer always has a curable intervention. The VOCs, released from urine and blood, are not so easily obtained like the ones from exhaled breath samples, but can also deliver a unique VOC-pattern in patients with ovarian, bladder and prostate cancer.

Unlike cancer diseases, metabolic and genetic disorders are even perceived by human olfaction. Most disorders of amino acid metabolism are diagnosed in the early childhood, thanks to their peculiar and unusual smells.

Similarly, patients suffering from kidney failure are also known for their ammonia-like urine smell, as well as diabetics for their acetone-like urine smell.

The VOCs released from faeces might play a significant role in diagnosing infectious diseases of the gastrointestinal tract, when detected and analysed by GC-MS, thus identifying VOCs characteristic of bacterial infections. Some other infectious diseases, such as *Mycobacterium tuberculosis* and viral infections are also well known for causing the production of diagnostic odours in afflicted patients, such as a distinctive stench odour in smallpox, known to physicians’ centuries ago.

In recent years a great number of studies have also been undertaken exploring the VOC-profiles in the exhaled breath of patients with cardiovascular diseases, asthma bronchiale, COPD and schizophrenia, suggesting a specific VOC-profile exists in these diseases, too.

Throughout this paper one could see that trained dogs are able to distinguish breath and urine samples of patients, diagnosed with cancer diseases from healthy controls with great accuracy and might also be used as future diagnostic tools.

However, their use in clinical practice is very limited when the costs, space and training sniffer dogs are taken into consideration.

Therefore, the standard equipment for detecting and analysing the VOCs consisted almost entirely of GC and GC-MS. In the past 20 years, the application of electronic noses has also come into widespread use.

With the introduction of NA-NOSES, which are much more sensitive than classic electronic noses, VOCs, characteristic of ovarian carcinoma, mesothelioma and COPD have been detected and quantified.

Despite all the progresses and advantages in this analytical equipment, many problems in using VOCs as diagnostic biomarkers still exist.

The analytical equipment is expensive and most techniques are time-consuming. One of the major problems is that there have not been sufficient trials within the operating practices of hospitals and other medical-care facilities.

Perhaps, the largest problem is a lack of information on biological pathways that produce the relevant VOCs in patients with the above mentioned diseases. For example in diseases, such as lung cancer or breast cancer, VOCs are released due to a lipid peroxidation or due to oxidative stress, but in many other diseases the biological pathways are still unknown, thus leaving no information on the origins, physiological and exhalation kinetics of the VOCs.

In the future it will be necessary to pay close attention to investigating the biological pathways, as they might be useful not only in diagnosing and monitoring human disease as biomarkers, but also in providing pathological mechanism of diseases and also in developing novel therapy.

Only when a lot more information and clinical trials on the VOCs have been obtained, will it be possible to apply them as routine, non-invasive, diagnostic biomarkers.

Since almost 80 % of all studies on the VOCs have been published in the last 5 years, further and intensive investigations in assessing that information are probably underway.

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9. CURRICULUM VITAE

Persönliche Daten:	Fanis Buljubasic
Geburtsort:	Tuzla
Staatsbürgerschaft:	Bosnien-Herzegowina
Studium:	Medizinstudium an der Medizinischen Universität Wien 2001-2005. Promotion zum Dr. med. univ. am 02.12.2005. Promotionsthema: "**Interleukin-13 promoter gene polymorphism-1112C/T is associated with the systemic form of mastocytosis**" Studienaufenthalte an der medizinischen Fakultät der Universität Heidelberg im SS 2003 und an der medizinischen Fakultät der LMU München im WS 2004.
Ausbildung:	Assistenzarzt auf der Kardiologie im Herzzentrum Leipzig 2008 -2009 Assistenzarzt in der 3. Medizinischen Klinik der Universität Rostock – Abteilung Hämatologie und Onkologie 2009- 2011 Assistenzarzt in der Klinik für Innere Medizin A der Universität Greifswald- Abteilung Gastroenterologie und Ernährungsmedizin 2011-2012
Immatrikulation:	Studium der Pharmazie an der Universität Wien, Oktober 2007.