University of Latvia

Faculty of Medicine, Medical Doctor Degree Program

Chair of Oncology

Volatile organic compound emission from gastric cancer tissue and its potential biological relevance

Diploma Thesis

Author: Dan Erik Aronsson

Student number: da11810

Supervisor: Prof. Mārcis Leja, MD, PhD.

Riga, 2017

TABLE OF CONTENTS:

ABBREVIATIONS

3-MST	3-mercaptopyruvate sulfur transferase
ALA	Aminolaevulinic acid
AN	Acrylonitrile
COPD	Chronic Obstructive Pulmonary Disease
CS_2	Carbon disulfide
CSE	Cystathionine-ϒ-lyase
CYB	Cystathionine β-synthase
CYP450	Cytochrome P-450 complex
GC-MS	Gas chromatography-Mass spectrometry
GST	Glutathione-S-transferase
H_2S	Hydrogen sulfide
KMTB	α-keto-ϒ-methylthiobuturate
MTP	3-methylthiopropionate
OAA	Oxaloacetate
PBG	Porphobilinogen
PPAR-α	Proliferator-activated receptor alpha
ROS	Reactive Oxygen Species
VOC	Volatile organic compound
VSC	Volatile sulfur compound
WHO	World Health Organization
ADH	Alcohol dehydrogenase
PPAR-α	Proliferator-activated receptor alpha
FFA	Free fatty acid
NMR	Nuclear magnetic resonance spectroscopy
GBH	Gamma-Butyric acid
GABA	Gamma-aminobutyric acid
GBL	Gamma-butyrolactone
MVP	Mevalonate pathway

UFT	Fluoropyrimidines
VEGF	Vascular endothelial growth factor
CNS	Central nervous system
LOD	Limit of detection
LOQ	Limit of quantification

ABSTRACT

Background: Volatile organic compound (VOC) emissions in exhaled air is an evolving field of research with regards to cancer diagnostics. VOCs are compounds that have a high vapor pressure under room-temperature conditions, leading to them evaporating into its gaseous form, which can be detected in all body tissues and liquids. The most research on the topic of VOCs in relation to cancer has been done on lung cancer. However, more studies are being done in determining the potential of VOCs in gastric cancer screening. Due to the complexity of VOC interactions with other metabolites from cells, the environment, microbes, etc., ascertaining their exact origin can be difficult. This study will try to investigate the potential origins of the compounds found in our specimens.

There are some different opinions on the importance of determining the VOC origins as long as they have a potential use in being able to differentiate a healthy versus an unhealthy state. Once you accumulate enough studies on VOCs related to a certain disease, the potential for detecting similarities in those studies are high. This can lead to the detection of biomarkers, or "fingerprints" for that disease. To understand why some VOCs are significantly increased in the cancerous tissue compared to the healthy tissue, the metabolic pathways of cancer cells become an important factor.

Objectives: VOC detection in the exhaled air has a potential for gastric cancer screening. Questions regarding the VOC origin and their biological relevance still require to be answered. This study aimed to identify substances being expressed in different concentrations between gastric cancer and non-cancerous tissue using GC-MS headspace analysis, as well as investigating potential source of origin of the VOCs released.

Methods: This study used tissue samples of 43 patients that were gathered during surgery. Two samples were obtained from each patient, one cancerous and one healthy. The size of the samples was on average 100mg (80-120mg). They were then stored in dry ice and analyzed with headspace needle trap extraction gas chromatography mass spectrometry (HS-NTD-GC-MS). The storage containers were sealed glass vials and the VOCs emitted into the headspace gas were transferred using a constant flow of ultra-pure air into the needle trap devices for extraction. The extracted analytes were then desorbed into the GS-MS system.

Statistical analysis: Wilcoxon signed-rank test was used with a confidence interval of 95% (p-value 5%).

Results: After excluding hospital environment related contaminants, a total of 32 VOCs were found to be produced by either type of samples. Emission of four of them (CS_2, pyridine, 3-methyl-2-butanone and 2-pentanone) was found to be significantly higher from cancer tissue when compared to the non-cancerous tissue, while another four (dimethyl sulfide, isoprene, butyrolactone and benzene) was decreased.

This study also investigated the significant volatiles to try and determine the origin and biochemical mechanisms involved in their production. CS_2, pyridine, 3-methyl-2-butanone and 2-pentanone can all be emitted from microbes. CS_2 can also be absorbed in trace amounts from environmental pollution. However, due to the significant differences in emission masses from these VOCs released between the two tissue samples, it is highly indicative that these VOCs are of cellular origin. CS_2 could be linked to cystine and cysteine metabolism and oxidative species interaction with cellular protein complexes, while both ketones seem to come from β-oxidation of fatty acids. This study was unable to link a cellular metabolic pathway or origin to pyridine, though it has been associated with periodontal disease and lung cancer. Isoprene has been found to be decreased in both lung and gastric cancer. Its origin seems to stem from the mevalonate pathway, which (aside from cholesterol synthesis) is involved in several regulatory functions on oncogenes in the Ras family. Benzene has been linked to environmental pollution and smoking, and is a known carcinogen. Gamma-butyrolactone was not found in previous cancer studies, but it could be related to fluoropyridine chemotherapy use. Dimethyl sulfide has been associated with lung and hepatocellular carcinoma and could also originate sulfur containing amino acids. Why it was decreased in the cancerous tissue, however, is still not clear.

Conclusion: The presence of certain VOCs may allow differentiation between cancer tissue and non-cancer tissue. The obtained data provide additional promising knowledge on the potential of VOC detection in gastric cancer diagnostics. The origin of the VOCs could be related either to the metabolism in cancer cells, oxidative stress, microbiome that also could be linked to the cancerous process and/or exogenous pollutants.

Key words: Gastric cancer, volatile organic compounds, head space analysis, tissue, GC-MS

KOPSAVILKUMS

Gaistošā organiskā savienojuma (VOC) emisija, ko var novērot izelpā, ir strauji augoša diagnostikas metode audzēju izmeklēšanā. VOC ir savienojums, kam istabas temperatūrā ir augsts tvaika spiediens, kas veicina tā iztvaikošanu gāzes formā. Šo gāzi var noteikt cilvēka audos un šķidrumos.(Haick et al 2014) Galvenokārt ir pētīta VOC saistība ar plaušu vēzi. Taču aizvien vairāk tiek veikti pētījumi, lai noteiktu, kādas ir iespējas VOC pielietot arī kuņģa audzēja gadījumā. Tā kā VOC molekula spēj mijiedarboties ar šūnu metabolītiem, apkārtējo vido, mikrobiem u.c., ir grūti saprast tā rašanās vietu. Šajā pētījumā tiks izmeklētas potenciālās savienojuma vietas, no kurām tas cēlies.

Ir dažādi viedokļi par to, vai ir nepieciešams atrast VOC izcelsmes vietu, kamēr tie spēj atšķirt veselu no nevesela stāvokļa. Ja tiktu apkopoti pietiekami daudz pētījumi par VOC saistību ar konkrētu slimību, tad būtu liela iespējamība atrast sakarības šajos pētījumos. Tas var veicināt biomarķieru atrašanu, kādai konkrētai slimībai. Lai saprastu, kāpēc daži VOC ir stipri vairāk audzēja audos nekā veselos audos, ir jāsaprot, ka audzēju šūnu metaboliskās īpatnības ir nozīmīgs faktors.

Objektīvi: Vai VOC noteikšana izelpā ir potenciāla metode kuņģa audzēja skrīningā. Vēl joprojām nav skaidrības par VOC izcelsmi un to bioloģisko būtiskumi. Tika mēģināts identificēt substances koncentrācijas atšķirību starp kuņģa audzēju un veselajiem kuņģa audiem, izmantojot *GC-MS headspace* analīzi.

Metode: Pētījuma tika izmantoti 43 pacienta audi, kas tika savākti operācijas laikā. No katra pacienta tika paņemti divi audu veidi – audzēja audi un veselie audi. Vidēji paraugs bija 100mg (80-120mg) smags. Tie tika uzglabāti sausā ledū un analizēti ar *headspace* adatas ekstrakcijas gāzes hromatogrāfijas masas spektrometru (HS-NTD-GC-MS). Kā uzglabāšanas konteinerus izmantoja aizzīmogotus stikla flakonus, izdalītā VOC gāze tika savākta caur adatu, izmantojot ļoti tīra gaisa nepārtrauktu plūsmu, kur tā nokļuva ierīce, kas ļāva to ekstrahēt. Savāktais ekstrakts tika novadīts uz GS-MS sistēmu.

Statistikā analīze. *Wilcoxon* tests tika izmantots ar 95% ticamības intervālu (p- vērtība 5%).

Rezultāti: Pēc tam, kad tika izslēgtas ar slimnīcas vidi saistītas izmaiņas, kopā tika atlasīti 32 VOC paraugi, kas tika producēti no viena vai otra parauga. Tika konstatēts, ka četriem no paraugiem CS2, piridīns, 3-metil-2-butanons un 2-pentanons bija stipri lielākā koncentrācijā

audzēju šūnas, salīdzinot ar veseliem audiem, kamēr citos četros paraugos bija samazināta dimetilsulfīda, izoprēna, butirolaktona un benzola koncentrācija.

Šajā pētījumā tika pētītas galvenās gaistošās vielas, lai pārbaudītu un noteiktu to izcelsmi un to ražošanā iesaistītos bioķīmiskos mehānismus. CS_2, piridīnu, 3-metil-2-butanonu un 2-pentanonu var iegūt no mikrobiem. CS_2 var arī absorbēties vides piesārņojuma mikroelementos. Tomēr, tā kā šo atbrīvoto GOS (gaistoši organiski savienojumi) emisiju masas ievērojami atšķiras šajos divos audu paraugos, tas skaidri norāda, ka šiem GOS ir šūnu izcelsme. CS_2 var sasaistīt ar metionīna, cistīna un cisteīna vielmaiņu, un oksidatīvās sugas mijiedarbojas ar šūnu olbaltumvielu kompleksiem, bet šķiet, ka abi ketoni rodas taukskābju beta-oksidācijas procesā. Pētījumā neizdevās sasaistīt šūnu vielmaiņas ceļu vai izcelsmi ar piridīnu, kaut arī to asociē ar periodontālām slimībām un plaušu vēzi. Ir konstatēts, ka izoprēna līmenis ir pazemināts gan plaušu, gan kuņģa vēža gadījumā. Šķiet, ka tā izcelsme rodas no mevalonāta ceļa, kas (papildus holesterīna sintēzei) ir iesaistīts vairākās Ras ģimenes onkogēnu regulējošajās funkcijās. Benzols ir sasaistīts ar vides piesārņojumu un smēķēšanu un ir vispārēji zināms kancerogēns. Gamma-butirolaktons netika konstatēts iepriekšējos vēža pētījumos, tomēr to ir iespējams saistīt ar fluorpiridīna izmantošanu ķīmijterapijā. Dimetil sulfīdu asociē ar plaušu un hepatocelulāro karcinomu un to var radīt arī sēru saturošas aminoskābes. Tomēr joprojām nav zināms, kādēļ tā līmenis vēža audos bija pazemināts.

Secinājumi: VOC var diferencēt audzēja audus no veselajiem audiem. Savāktie dati sniedz papildus zināšanas par VOC potenciālu kuņģa audzēja diagnostikā. VOC izcelsme var būt saistīta ar audzēja šūnas vielmaiņu, ar oksidatīvo stresu, vai ar mikrobiomu, kas arī varētu būt saistīts ar audzēja procesu un/vai eksogēniem piesārņotājiem.

1. INTRODUCTION

Per the WHO, gastric cancer is currently the third most common cause of cancer-related death in both sexes worldwide *(WORLD HEALTH ORGANIZATION FACT SHEET (2017).* Gastric cancer is usually asymptomatic in early stages of disease, and is therefore diagnosed in advanced stages were the prognosis is poor. *(Krilaviciute et al. 2015)* With population based screening, one can improve the prognosis and reduce mortality, but there are currently no such effective population based screening methods available. The best available method to date, is pepsinogen detection through serum analysis. This method still lacks the required sensitivity to be applicable as a screening test, and thus cannot be recommended by itself for population based screening *(Leja et al. 2017).*

Currently the gold standard for diagnosis and screening of gastric cancer is endoscopy followed by a biopsy and histopathological evaluation. However, endoscopy is not considered a suitable method for population-based screening due to its invasiveness, high cost, and the need for experienced personnel to perform an endoscopic procedure. The use of endoscopy as a screening method is still controversial even in areas of high incidence of gastric cancer (such as in Eastern Asia). *(Chan 2017)* Other available tools include barium swallow and detection of micro-MRNAs in the serum or gastric aspirate. Barium swallow has been shown to be inferior to endoscopy in both sensitivity and specificity, and the latter method still require further research to support its use. Additionally, the ability to perform a biopsy during an endoscopic procedure adds much more value to its clinical utility when compared to the other approaches *(Mansfeld 2017 and Chan 2017).* Due to the cumbersome nature of endoscopy and its inconvenience to the patient, a highly accurate, non-invasive screening method for gastric cancer and related precancerous lesions is still needed.

A rapidly developing field of research is to use breath analysis to try and detect various types of cancer. Several studies have found a correlation between the detection of certain volatile organic compounds (VOCs) in exhaled breath, or from tissue analysis, and cancer.

1.1 Volatile organic compounds

VOCs are organic compounds that have a high vapor pressure under ordinary room temperatures. This pressure is due to their low boiling point, which cause large numbers of

molecules to evaporate from the liquid or solid form. VOCs are emitted constantly from every cell in the human body. Most scents and odors are due to VOCs. Different cell lines seem to produce some differences in VOCs. This could be due to the differences in function, and thus, cellular metabolism in the different cell lines. VOCs emission in cells is thought to be a byproduct of cellular metabolism. The question is, can these emissions be used in the diagnosis of gastric cancer?

1.2 Hypothesis

The following hypothesis were set:

- VOC profiles can differentiate between cancerous tissue and healthy tissue by measuring VOC emission from tissue in headspace analysis.

1.3 Objectives

The aim of this work was to:

- To evaluate the VOC profile emitted in the headspace of cancerous tissue versus healthy tissue.

- To try and determine the possible origin of the VOCs found.

1.4 Tasks

The following tasks were set:

- To obtain cancerous and healthy tissue specimens from 43 subjects during surgical procedure

- To measure the VOC profile emitted from the tissue samples using gas chromatography-mass spectrometry on the VOCs emitted in the headspace of those tissues.
- To compare the findings with other studies done on VOCs and cancer

- To try to determine the origin of the VOCs emitted

- To try to assess the uses and practicality of VOC emission in cancer diagnostics

2. LITTERATURE REVIEW

2.1 GASTRIC CANCER

Gastric cancer is still one of the leading causes of cancer related death worldwide, regardless of decreasing incidence levels due to improved diet, improved food preparation and the discovery of *H. pylori.* Its prognosis is still poor, with overall survival rates ranging from 5-15% in the USA and other Western countries. Japan has one of the highest incidences of gastric cancer in the world, and in the 1970s they implemented a screening program which dramatically improved the 5-year survival rate. This is not routine in most other countries and so gastric cancer is usually diagnosed at advanced stages of disease. Also, endoscopic screening comes at a cost and with non-negligible morbidity potential. Gastric cancer is diagnosed more frequently in men with around 2:1 incidence ratio, and has a peak incidence around 60-70 years of age in men, and a slight later peak incidence in women *(Karpeth et al. 2001, Chu et al. 2015).*

Gastric malignancies consist of different histological subtypes including, adenocarcinoma, carcinoid tumors, lymphomas, gastrointestinal stromal tumors and leiomyosarcomas. Around 95% of all malignant gastric cancers are adenocarcinomas. Staging, location and histological subtype is the major factors determining treatment approach in gastric cancer. *(Chu et al. 2015)*

2.1.1 Current approaches used in diagnosing gastric cancer

Cancer diagnosis involves the confirmation of disease and disease staging. To achieve this a biopsy is always required. Esophagogastroduodenoscopy has a diagnostic accuracy of around 95%. This procedure is also the main method used to obtain a tissue diagnosis of suspected lesions. In any lesion, a biopsy should include six specimens taken from around the lesion due to variable malignant transformation. Therefore, endoscopy is still the gold standard for gastric cancer diagnosis, followed by histopathological evaluation. Other diagnostic tools available are CT- scans, blood tests, MRI, barium swallows, measuring serum pepsinogen and/or serum trefoil factor-3 and the detection of micro-MRNAs in the serum or gastric aspirate. Barium swallow has been shown to be inferior to endoscopy in both sensitivity and, and the latter three still require more research to support their use. *(Chan 2017)*

2.2.2 Volatile organic compound emission for cancer diagnosis

In 2011 *Sonoda et al. (2011)* did a study where they trained a Labrador retriever to be able to detect colorectal cancer using scent. After training the dog for 4 years using a reward based approach, the Labrador was tested to see if he could identify those with colorectal cancer from breath and stool samples. The experiment was performed in such a way that a cancer sample was placed in a room with 4 control samples. The detection of colorectal cancer in exhaled breath using this method had a sensitivity of 91% and a specificity of 99%. In the stool sample, the sensitivity was 97% and specificity 99%. This study indicated that the trained Labrador could somehow discriminate patients with colorectal cancers using scent with a very high diagnostic accuracy.

Another study related to canine olfaction and cancer was done by *Ehmann et al. in 2012*. They used four dogs, two German shepherds, one Labrador and an Australian shepherd of both sexes, two males and two females, for scent detection of lung cancer. They collected breath samples from 220 subjects and the dogs could identify lung cancer with a sensitivity of 71% and specificity of 93%. The detection of lung cancer in these participants was independent of Chronic Obstructive Pulmonary Disease (COPD), smoking and food odors. It is, however, not very practical to use canines for diagnostic purposes in day to day hospitals and clinics as the facilities are not necessarily appropriate. Nevertheless, the importance of those studies does support the theory that specific VOC profiles for cancer seem to exist, and could potentially be identified with newer modern techniques.

VOCs released from cancer cells originate from the cells or the disease location. They are believed to be produced due to the alterations of specific biochemical pathways in the body, most which are linked with oxidative stress, cytochrome p450, liver enzymes, carbohydrate metabolism and lipid metabolism. *(Broza et al 2014)* They then enter the surrounding environment. This creates one potential difficulty in using VOCs for diagnosis as the number of VOCs found using GC-MS from a tissue sample can be very large. When you consider the VOCs produced by cellular metabolism, the VOCs found in atmospheric air, the VOCs produced by the billions of microbes in your gut flora as well as each separate VOCs potential ability to react with one another, it can become very complex.

VOCs released from cancer cells can be identified from:

1. The headspace of cancer cell lines
2. Exhaled breath

3. Urine
4. Plasma
5. Skin
6. Faeces

(Broza et al. 2014, Haick et al. 2014)

Current data seems to point to different cells and tissues having a specific volatile profile depending on the tissue or organ, and that it is highly unlikely that a single VOC biomarker would be able to differentiate cancers subjects from the control groups. *(Kumar et al 2014)* The origin of these volatiles is still relatively unclear even though several theories have been raised. Two exceptions are isoprene and acetone, where the biochemical pathways can be given. Therefore, the exploration of several VOC profiles and patterns in VOC emission is more likely going to lead to a potential "fingerprint" for that disease.

This study was performed by taking tissue samples from the gastric mucosa and measuring the VOCs from the headspace of that tissue sample.

2.2 IMPORTANT FACTORS TO CONSIDER IN RELATION TO VOCS AND CANCER

VOC emission is multifactorial. In *Ahn et al.' (2016)* study they showed that radiation of methionine alone produced 12 different VOCs. This is because the VOC produced depends largely on where the chemical bonds are broken in the methionine molecule, as well as which other VOCs are present in the immediate environment leading to secondary reactions. To understand the relationship between cancer and VOC emission it is important to look at cancer cell metabolism, as well as the role of oxidative stress, exogenous pollutants and the gut microbiome.

2.2.1 Metabolism in cancer cells.

One very famous study on cancer metabolism was done by Otto Warburg in the 1920s. Glycolysis is a physiological response to hypoxia in normal tissues, but Otto Warburg observed that tumor cells take up glucose and produce lactate regardless of oxygen availability. This has been seen in many types of cancer cells and has been validated using fluorodeoxyglucose positron emission tomography. Fluorodeoxyglucose uses a radioactive

glucose molecule to provide an image of glucose uptake in tumors and tissue. *(Koppenol et al. 2011)* Warburg's hypothesis did, however, also lead to a widely-held misconception that cancer cells rely on glycolysis as their major source of ATP. It is now clear that cancer cells can use aerobic glycolysis due to activation of oncogenes, loss of tumor suppressor genes and up regulation of the phosphatidylinositol-3-kinase (PI3K) pathways. Several studies have now demonstrated that most cancer cells can produce energy through glucose oxidation (the process were glucose-derived carbons are oxidized to CO2 to produce ATP from oxidative phosphorylation), fatty acid oxidation and amino acid metabolism. *(Deberardins et al. 2016)* This is also supported by other studies were inhibition of enzymes in the glycolysis pathway failed to inhibit tumor growth. *(Israelsen et al. 2013)*

Mitochondrial metabolism is needed for cancer cells to proliferate. So, despite of their high glycolytic rate, the majority of cancer cells generate most of their ATP from the mitochondria. In addition to pyruvate derived from glycolysis, amino acids and fatty acids can supply substrates into the citric acid cycle to maintain ATP synthesis in cancers cells. β-oxidation of fatty acids in the mitochondria produces acetyl-CoA and the reducing equivalents NADH and FADH, which can be used by the electron transport chain to produce ATP. *(Deberadins et al. 2016)* Per *Abassi et al's. 2013* review on metabolomic profiling of gastric cancer they state that fatty acid β-oxidation has been confirmed as a dominant pathway for energy generation in numerous malignancies including prostate and pancreatic cancer. Ketones and aldehydes, which are metabolic products of β-oxidation, are shown to be increased in biological samples of patients with oeasophago-gastric (OG) cancers.

Abassi et al. (2013) also state that local inflammation and oxidative stress associated with cancer influences the synthesis of eicosanoids from the plasma membrane and FFAs. This oxidative stress also induces lipid peroxidation, which leads to the production of aldehydes.

Amino acids are another source of fuel that tumor cells use. Glutamine can be converted to glutamate and then subsequently to α-ketoglutarate. α-ketoglutarate can be used to fuel the citric acid cycle via glutaminolysis. Isoleucine, valine and leucine can also be converted into acetyl-CoA and other organic molecules to enter the citric acid cycle *(Mayers et al. 2014 and Hensley et al. 2013*). *Deberadins et al. 2016* suggests that a combination of the local tumor environment and oncogenic lesions is likely to dictate the fuel used by mitochondria to maintain cancer growth. The accessibility to nutrients for tumor cells depends largely on their proximity to the vasculature. The cells that are closest to the vasculature can use nutrients and

oxygen to fuel growth, while the cells distant from the vasculature have less access to nutrients and oxygens and thus may use other forms of metabolism such as fatty acid oxidation and amino acids metabolism

2.2.3 Oxidative stress

One very important factor in the development of cancer is associated to increased oxidative stress and the induction of the oxidase enzymes of the cytochrome p-450 complex (CYP450). The oxidative stress occurring in the organism is due to the balance between the formation and deactivation of reactive oxygen species (ROS) and free radicals. During the various oxidative processes (oxidative phosphorylation, β-oxidation etc.) occurring in the mitochondria, cells produce ROS all the time. Some ROS include superoxide radical, hydrogen peroxide, hydroxyl anion, peroxinitrite, etc. ROS have an unpaired electron in the outer shell and is thus highly reactive. Other sources of ROS could also be from exogenous origins such as cigarette smoke, pollution, drugs and radiation. *(Haick et al. 2014)*

Once ROS become accumulated and there is a disequilibrium between a cells antioxidizing capability and the generation of ROS, these highly reactive molecules can react with several structures in their environment such as lipids and proteins. It is believed that these “attacks” induced by free radicals could be part of the process in VOC formation as will be explained further later.

2.3 THE ROLE OF MICROBIOME

The gastrointestinal tract is composed of 100 times as many genes as the entire human genome. It is believed to contain between 500 to 1000 species and contains 10 times more cells than the entire human body. *(Sagar et al. 2015, Quigley 2013)* The gut microbiome is also now known to have a great impact on overall homeostasis of the body, and it is seeming to have a significant role in several immunologic interactions. Some important functions of the gut microbiome include:

- Metabolic role in producing short-chain fatty acids, vitamin K and folic acid synthesis, production of arginine and glutamine and salvaging calories.
- Deconjugates bile acids
- Protective role in preventing pathogens from colonizing

- Immunologic effects by stimulating Immunoglobulin A (IgA) production, promoting up-regulation of anti-inflammatory cytokines while down regulating pro-inflammatory cytokines.
- Promotes regulatory T-cells

(Quigley (2013)

There has been a lot of interest regarding microbes in relations to VOCs, and their influence on the results found in several cancer studies. Which VOCs are produced by which microbes? What kind of interactions occur between the volatiles produced by the human organism and the microbes?

It is already known that bacteria can produce a wide range of VOCs. As mentioned earlier, many VOCs are believed to come as a result of cellular metabolic processes, or as by-products of metabolic processes. Several studies have been done with regards to identification of VOCs produced by microbes, as well as studies that try to determine metabolic pathways used by the microbe that lead to the VOC emission. (*Tait et al. 2014)* One study has also tried to use VOC profiles in determining the health of the gut flora. *(Sagar et al. 2015)*

Due to the vast number of microbes found in the intestinal flora it is important to consider their potential role in the VOC profiles found. Our samples were taken from the stomach were the main inhabitants are *Staphylococcus, streptococcus, peptostreptococcus, lactobacillus* and some yeast types *(Sherwood et al. 2013*). *Helicobacter pylori (H. Pylori)* is also present in around 50% of the population. *(Amieva et al. 2016)*

2.4 ASSESSING THE ORIGIN OF VOLATILE ORGANIC COMPOUNDS IN CANCER

Metabolic changes occur all the time, both in the normal physiological state and during abnormal metabolic processes that can occur due to disease. It is believed that during diseased states, these abnormal processes can change the body's chemistry and that it can alter the VOC concentration emitted from that tissue (or cell), or produce new VOCs altogether. Other factors one will have to consider is the VOCs that are already present in atmospheric air, VOCs originating from exogenous sources (such as drugs, tobacco smoke, alcohol etc.) and VOCs that are emitted from the microorganism present in our organism. Different VOCs can also react with other VOCs in the environment to from even newer compounds. Another

factor that one should consider is the normal metabolism of each individual. For example, it could be possible that one person suffering from diabetes and gastric cancer could have a different concentration of certain VOCs when compared to a person only suffering with gastric cancer.

Origins that would need to be taken into consideration when trying to determine VOC origins of gastric cancer include:

1. Metabolic processes
2. Release from storage sites in the body
3. Oxidative stress
4. Microbiome
5. VOCs released from equipment used in the measurement
6. Interactions that occur between VOCs
7. Comorbidities in the patient
8. VOCs present in atmospheric air
9. VOCs present in the normal gastro intestinal tract

3. METHODS AND MATERIALS

3.1 Study design

This study was an experimental study performed in Riga East University Hospital, Latvian Oncology Centre, Institute of Clinical Medicine University of Latvia. The GC-MS analysis was performed in the Breath Research Institute, University of Innsbruck. All specimens were obtained at various intervals in 2016-2017.

3.2 Patients

A total sample of 43 patients diagnosed with various clinical stages of gastric cancer was used. The patient group consisted of 25 males and 18 females. 19 were confirmed smokers (or had been smoking prior to diagnosis) while 24 had never smoked.

3.3 Methods

All tissue samples were taken during surgery. One sample of cancerous tissue and healthy tissue were collected in parallel from each patient. The tissue samples were then frozen down to -86 ℃.

During the analysis, around 100 mg (+/- 10%) of frozen tissue was placed in a headspace vial (2 mL, Gerstel, Germany), that was then purified using purified air and closed with a silica septum. The sample was then defrosted at 37 ℃ for 30 minutes. During this phase, the evaporation of VOCs into the vial headspace is also stimulated. Two-bed 23-gauge stainless steel needle trap devices (NTD) (2 cm of Carbopack X and 1cm of Carboxen 1000, both 60/80 mesh, PAS technology, Germany) were used to extract the the VOCs released by the tissue samples. Before they were used all NTDs were pre-conditioned at 290°C by flushing them with a high-purity nitrogen flow (6.0 – 99.9999%) for 10-15min. The NTDs was inserted into the vial via a rubber septum, drawing 100 mL of the headspace gas at a steady flow rate of 2mL/min at 25 ℃. In order to keep a constant pressure, high purity air was introduced into the vials at a flow equal to sampling flow. After the extraction, the NTD was placed in to the GC were the compounds were desorbed at 290 ℃.

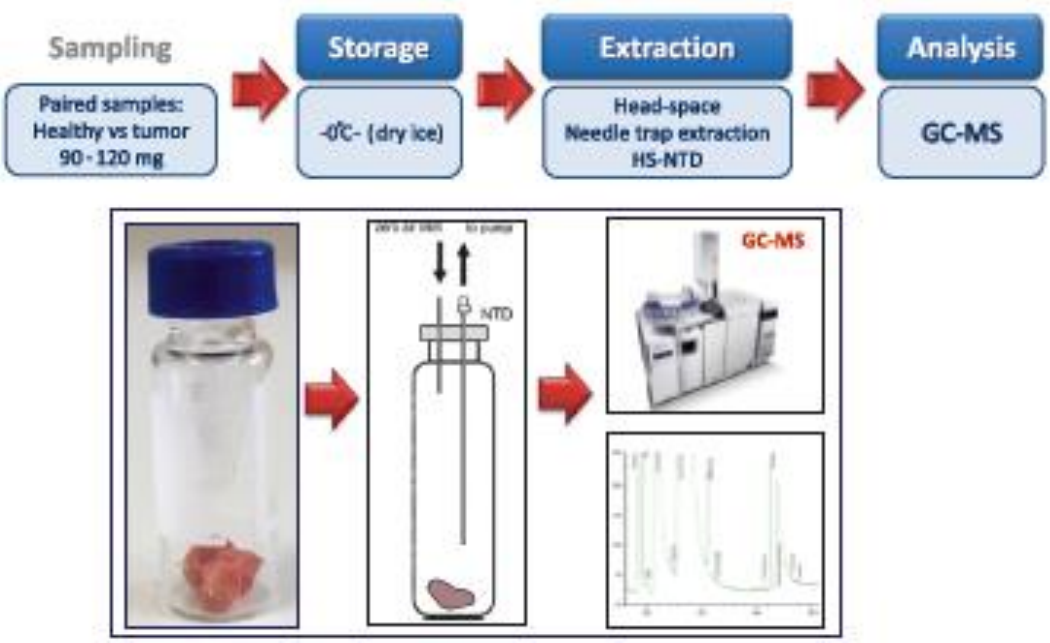


Fig. 1 Sequence of methodological process.

Chromatographic analyses were done by using an Agilent 780A/5975C GC-MS system. The VOCs were separated using an Rt-Q-BOND column (30mx0.25 m, film thickness 8 µm, 100% divinylbenzene phase, Restek, USA) working in a constant flow of helium at 1 $mLxmin^{-1}$. The start temperature was 40 ℃ for one minute, and then increased by 5 ℃ every min^{-1} until it reached a constant temperature of 210 ℃. After 4 minutes, this was then again increased by 3 ℃ min^{-1} to 260 ℃. A constant temperature of 260 ℃ was kept for 14 minutes.

The mass spectrometer was working in a combined SCAN/SIM mode. The SCAN, was set with an associated range from m/z 20 to m/z 200, and used for the untargeted analysis and identifying compounds emitted by the tissue samples as well as for the quantification of species that were more abundant. The peak integration was based on extracted ion chromatograms. The substance specific m/z ratios selected for this purpose allowed in numerous cases for a proper separation of compounds from their neighboring peaks, even when the latter were overlapping in the total ion count chromatogram. The applied SCAN quantifier ions are presented in Table 1. Selected less abundant species were quantified using SIM (selective ion monitoring mode), with the corresponding m/z ratios and dwell times

being presented in Table 1. The quadrupole, ion source, and transfer line temperatures were kept at 150°C, 230°C, and 280°C, respectively.

The VOC identification process was performed in two steps:

Firstly, the peak spectrum was compared against the NIST mass spectral library. Secondly the NIST identification was confirmed by comparing the respective retention times with the retention times obtained based on standard mixtures prepared from pure compounds. The VOC emission was quantified using calibration mixtures prepared from pure liquid or gaseous liquid whenever possible.

3.4 Statistical analysis

Wilcoxon signed-rank test was used to compare the two samples. A p-value of < 0.05 was put as significance value.

3.5 Ethical consideration

The study was approved by the Riga Eastern University Hospital, Medical and Biomedical appointed ethical committee, Latvia. All patients agreed that we were allowed to take the specimens during the surgical procedure. The patient data was coded so that no personal information was exposed. There was no extra risk brought on to the patient outside the normal risk of any surgical procedure. The specimens were exclusively used to measure their volatile profile using GC-MS.

4. RESULTS

4.1 Method validation

The calculated validation parameters are presented in Table 1. LOD is the lowest analyte concentration needed in order to determine that the VOC was present. The LOD values ranged from 0.01 to 1.23 pmol. The limit of quantification (LOQ) was defined as 3×LOD. Relative standard deviations (RSDs) were calculated on the basis of consecutive analyses of five tissue samples obtained from the same patient. The calculated RSDs varied from 6-30% and were deemed as acceptable for the goals of the study. It should be stressed that this parameter is affected by a small size of the samples available for the extraction and sample shape differences. The system response was found to be linear within the investigated concentration ranges (see Table 1), with coefficients of variation ranging from 0.913 to 0.999.

4.2 Emission of VOCs from gastric tissues

An Exemplary chromatogram from cancer and normal tissues HS-NTE-GCMS analysis is presented in Fig. 2. A total number of 45 compounds was found in the head-space of tissue samples. Excluding hospital environment related species and their metabolites (e.g. methanol, ethanol, 2-propanol, sevoflurane, hexafluoroisopropanol) and compounds with incidence below 20% 32 volatiles were detected in the head-space of both cancer and normal tissues. Their associated detection and quantification incidences as well as the observed emissions during experiments (in pmols) are given in Table 2. The predominant chemical classes were hydrocarbons and heterocyclics with six and five species, respectively. Apart from these, there were four ketones, three volatile sulphur compounds (VSCs), three nitriles, three amides, three aromatics, three aldehydes, one ester and one terpene. Eleven compounds (acetaldehyde, methanethiol, acetone, CS_2, isoprene, 2-butanone, 2-pentanone, pyrrole, pyridine, furfural, n-octane) were found in all samples. Emission of 22 species (69%) was quantified using the aforementioned procedures. The remaining compounds could not be quantified properly, either due to the unavailability of pure substances, or due to problems related to the preparation of reliable standard mixtures. Although detected in the head space of all tissue samples acetone was not quantified as its signal exceeded the dynamic range of the MS detector. The observed emission ranged from 0.17 pmol for 2-propenenitrile to 38 pmol for 2-butanone considering

medians. However, half of all quantified species exhibited emissions below 1 pmol. The highest median levels were noted for 2-butanone (38 pmol and 34 pmol for cancer and normal tissues respectively) and CS_2 (19.9 pmol for cancer tissue). A Wilcoxon signed rank test was used to compare the emissions of VOCs from cancer and non-cancerous tissues, and a p value of <.05 was considered as significant. Emission of four species (CS_2, pyridine, 3-methyl-2-butanone, 2-pentanone) was found to be significantly higher from cancer tissue, and further four (isoprene, butyrolactone, dimethyl sulphide, and benzene) were decreased in the cancerous tissue compared to the non-cancerous.

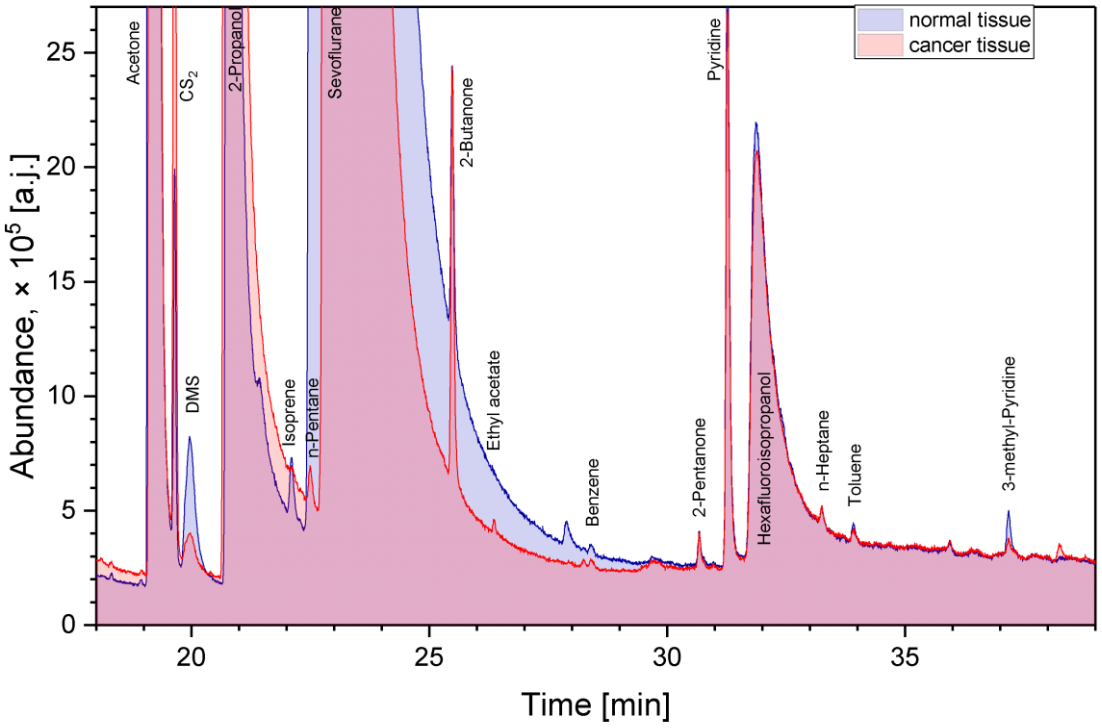


Fig 2. Shows a fragment of an example chromatogram from HS-NTE-GCMS analysis of cancer and normal tissues

VOC	CAS	R_t [min]	Quantifier ion (SIM dwell time [µs])	LOD [pmol]	RSD [%]	R^2	Linear Range [pmol]
Acetaldehyde	*75-07-0*	*11.52*	*44*	-	*20*	-	-
Methanethiol	*74-93-1*	*12.64*	*47*	-	*25*	-	-
1-Propene, 2-methyl-	*115-11-7*	*14.94*	*41*	-	*6*	-	-
Acetonitrile	75-05-8	16.83	41 (80)	1.23	15	0.913	3.7-46
n-Propanal	123-38-6	18.95	58	0.04	16	0.998	0.1-15
Carbon disulfide	75-15-0	19.65	76	0.06	18	0.999	0.2-38
Dimethyl sulfide	75-18-3	19.89	62 (40)	0.01	13	0.994	0.03-8.5
2-Propenenitrile	107-13-1	20.21	53 (80)	0.04	20	0.989	0.11-10
Isoprene	78-79-5	22.11	67 (80)	0.02	14	0.989	0.06-10
n-Pentane	109-66-0	22.48	72 (40)	0.3	10	0.990	0.9-23
2-Butanone	78-93-3	25.48	72 (80)	0.35	8	0.970	1-100
Ethyl Acetate	141-78-6	26.35	43 (80)	0.08	20	0.955	0.23-40
n-Hexane	110-54-3	28.30	43 (80)	0.05	12	0.986	0.15-20
Benzene	71-43-2	28.40	78	0.06	10	0.974	0.19-9
2-Butanone, 3-methyl-	563-80-4	29.69	43 (80)	0.05	17	0.996	0.15-10.5
2-Pentanone	107-87-9	30.68	43 (80)	0.06	7	0.981	0.19-35
Pyrrole	*109-97-7*	*30.78*	*67*	-	*20*	-	-
Acetamide	*60-35-5*	*31.11*	*59*	-	*30*	-	-
Pyridine	110-86-1	31.30	79	0.12	16	0.982	0.35-150
Pyrrole, 1-methyl-	96-54-8	31.36	81	0.06	20	0.971	0.18-8.6
n-Heptane	142-82-5	33.23	71 (80)	0.12	13	0.975	0.36-12.6
Acetamide, N,N-dimethyl-	*127-19-5*	*33.70*	*43*	-	*20*	-	-
Toluene	108-88-3	33.93	91	0.17	20	0.993	0.5-11
Furfural	98-01-1	34.68	96 (80)	0.17	12	0.971	0.5-10
Pyridine, 3-methyl-	*108-99-6*	*37.17*	*93*	-	*20*	-	-
Butyrolactone	*96-48-0*	*37.29*	*42*	-	*27*	-	-
n-Octane	111-65-9	38.27	43 (80)	0.05	12	0.986	0.15-20
p-Xylene	106-42-3	39.58	91	0.1	14	0.991	0.3-11
Benzonitrile	100-47-0	44.38	103	0.27	15	0.986	0.8-20
D-Limonene	5989-27-5	50.82	93	0.29	18	0.993	0.9-5
Benzamide	*55-21-0*	*65.56*	*121*	-	*30*	-	-

Table 1: Retention times R_t [min], quantifier ions, SIM dwell times [µs], LODs [pmol], RSDs (%), coefficients of variation (R^2) and linear ranges [pmol] of compounds under study. Compounds in italics were not quantified for reasons mentioned in the text. Compounds are ordered with respect to increasing retention time.

VOC	Cancer tissue		Normal tissue		p-value Wilcoxon test
	$n_d(n_q)$	Range (Median) [pmol]/*[-]*	$n_d(n_q)$	Range (Median) [pmol]/*[-]*	
Acetaldehyde	*43(43)*	*515-4200 (1130)*	*43(43)*	*360-12500 (1800)*	*n.s.*
Methanethiol	*43(43)*	*4-470 (34)*	*43(43)*	*6.6-250 (32)*	*n.s.*
1-Propene, 2-methyl-	*43(43)*	*10-2060 (27)*	*42(42)*	*11-3250 (31)*	*n.s.*
Acetonitrile	31(31)	3.7-110(7.2)	42(35)	3.7-78.7(6.8)	n.s.
n-Propanal	42(42)	0.8-16.6(2.2)	41(41)	0.9-8.3(2.6)	n.s.
Acetone	43(43)	-	43(43)	-	-
Carbon disulfide	43(43)	0.7-260(19.9)	43(43)	0.5-21(1.4)	8.2×10^{-4}
Dimethyl sulfide	40(40)	0.05-8.0(0.66)	41(41)	0.2-8.1(0.88)	0.029
2-Propenenitrile	43(20)	0.11-1.8(0.3)	41(18)	0.11-1.5(0.17)	n.s.
Isoprene	43(43)	0.22-15.7(1.3)	43(43)	0.64-15.7(2.1)	1.8×10^{-3}
n-Pentane	42(38)	1.0-57(2.5)	42(35)	0.9-10.6(2.3)	n.s.
2-Butanone	43(43)	5.5-246(38)	43(43)	6.9-152(34)	n.s.
Ethyl Acetate	42(42)	0.3-13.5(1.66)	42(39)	0.4-167(2.3)	n.s.
n-Hexane	31(23)	0.16-34.5(0.67)	33(31)	0.16-4.4(0.46)	n.s.
Benzene	43(36)	0.21-2.1(0.43)	38(38)	0.22-2.0(0.55)	0.046
2-Butanone, 3-methyl-	43(42)	0.15-5.5(0.63)	42(39)	0.15-2.64(0.54)	6.9×10^{-4}
2-Pentanone	43(43)	0.4-63.5(1.7)	43(43)	0.51-21(1.4)	3.0×10^{-3}
Pyrrole	*43(43)*	*10.6-243(27)*	*43(43)*	*10.3-229(24)*	*n.s.*
Acetamide	*36(36)*	*17-1740(66)*	*33(33)*	*20.5-696(66)*	*n.s.*
Pyridine	43(43)	0.62-685(7.7)	43(43)	0.58-135(3.0)	1.6×10^{-4}
Pyrrole, 1-methyl-	12(9)	0.74-5.0(1.5)	12(4)	0.62-1.6(1.3)	n.s.
n-Heptane	24(9)	0.4-22(0.66)	28(11)	0.48-2.7(0.74)	n.s.

Acetamide, N,N-dimethyl-	*34(34)*	*10-463(33)*	*38(38)*	*5.5-171(31)*	*n.s.*
Toluene	14(8)	0.55-0.8(0.59)	15(8)	0.56-4.6(0.69)	n.s.
Furfural	43(43)	0.33-5.4(0.72)	43(43)	0.28-3.43(0.81)	n.s.
Pyridine, 3-methyl-	*30(30)*	*5.7-224(21)*	*26(26)*	*3.5-263(16)*	*n.s.*
Butyrolactone	*42(42)*	*7.3-256(40)*	*43(43)*	*9.7-277(48.5)*	3.6×10^{-3}
n-Octane	43(39)	0.14-78(0.65)	43(35)	0.16-10.3(0.93)	n.s.
p-Xylene	33(27)	0.33-0.78(0.44)	30(26)	0.32-1.49(0.47)	n.s.
Benzonitrile	40(40)	0.97-7.7(2.1)	41(41)	0.71-10.1(1.84)	n.s.
DL-Limonene	35(12)	0.95-1.12(1.0)	32(7)	0.88-1.21(1.15)	n.s.
Benzamide	*29(29)*	*8.6-956(42)*	*29(29)*	*4.9-364(39)*	*n.s.*

Table 2: Detection (n_d) and quantification (n_q) incidences of the compounds under study, their emission ranges [pmol] and the outcome of a Wilcoxon signed rank test. (n.s. – not significant) For uncalibrated species (in italics) ranges and medians of peak areas are provided.

The GS-MS analysis was performed at the University of Innsbruck. The personal contribution of the author was to analyze all the significant VOCs by interpreting the results and determine potential sources of origin as well as potential metabolic and biochemical pathways.

COMPOUNDS INCREASED IN CANCEROUS TISSUE	p-value	COMPOUNDS DECRESED IN CANCEROUS TISSUE	p-value
Carbon disulfide (CS2)	8.2×10^{-4}	Isoprene	1.8×10^{-3}
2-Pentanone	3.0×10^{-3}	Gamma-Butyrolactone	3.6×10^{-3}
3-Methyl-2-Butanone	6.9×10^{-4}	Benzene	0.046
Pyridine	1.6×10^{-4}	Dimethyl Sulfide	0.029

Table 3: Outlining of VOCs found to be statistically significant in cancerous tissue samples when compared to the non-cancerous samples.

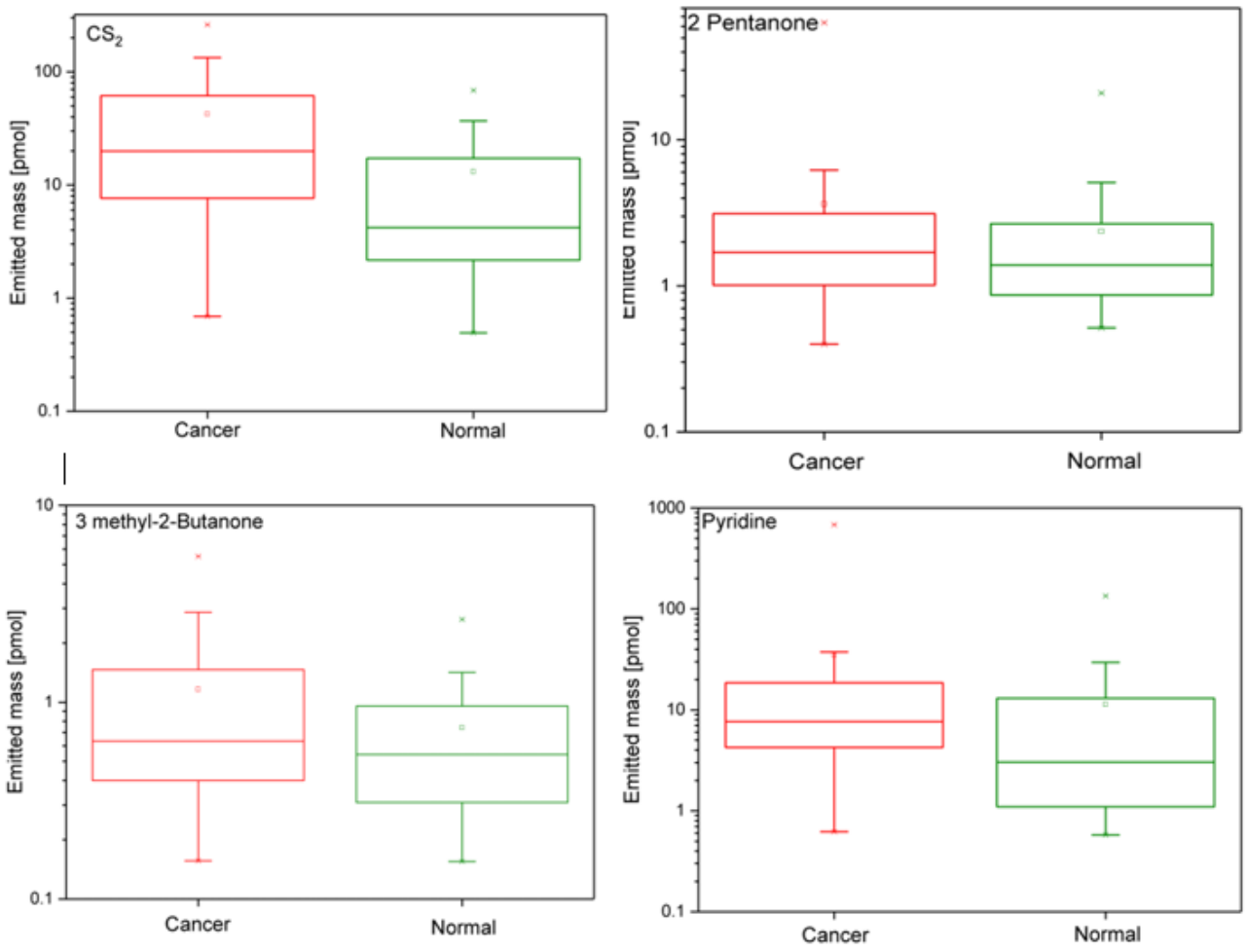


Fig. 3 Comparison of emissions of CS_2, pyridine, 2-pentanone and 3-methyl-2-butanone from cancerous and non-cancerous tissue.

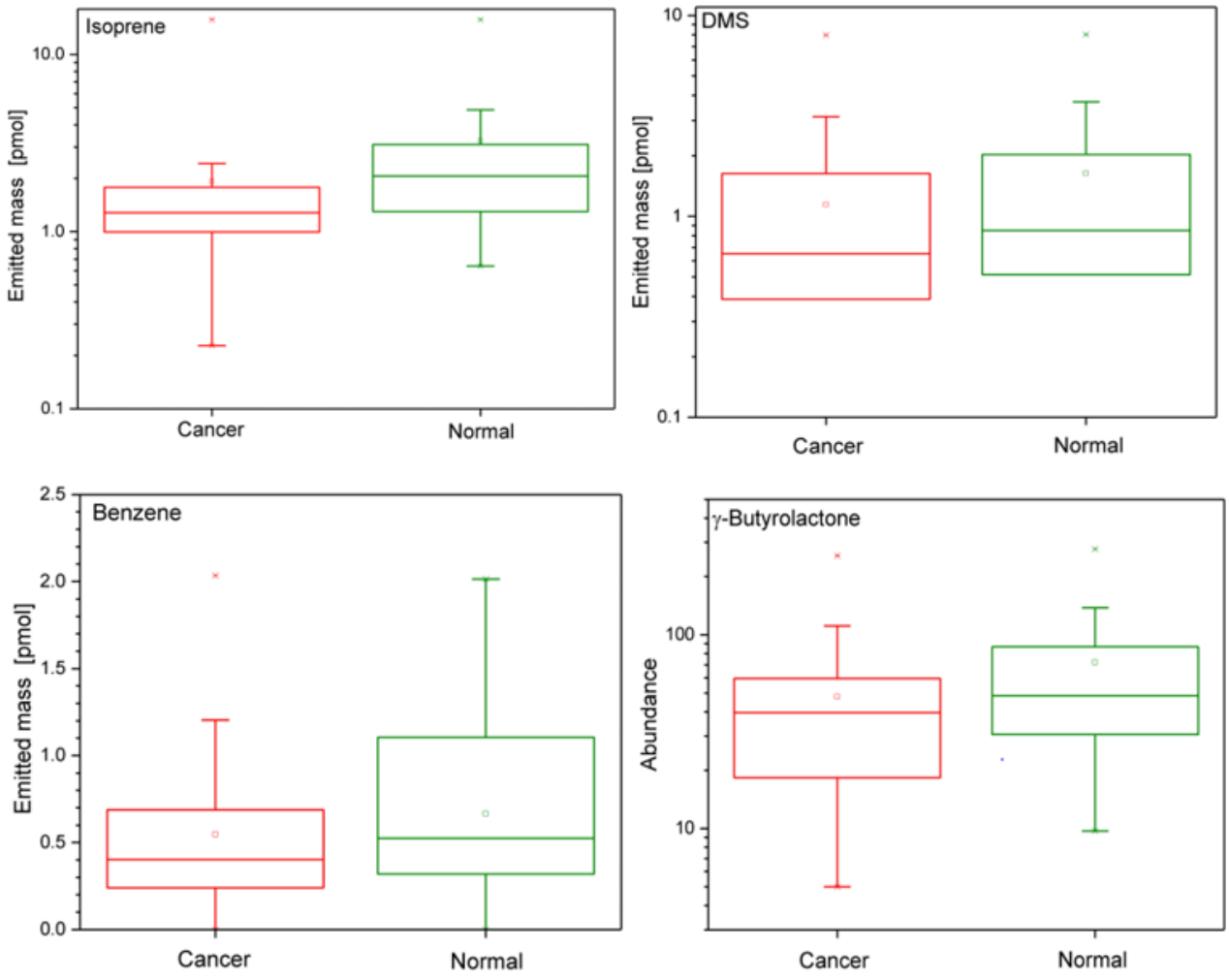


Fig. 4 Comparison of emissions of isoprene, dimethyl-sulfide, benzene and gamma-butyrolactone from cancerous and on-cancerous tissue.

5. DISCUSSION

5.1 Carbon disulfide (CS2), Methanethiol (Mercaptomethane) and Dimethyl sulfide.

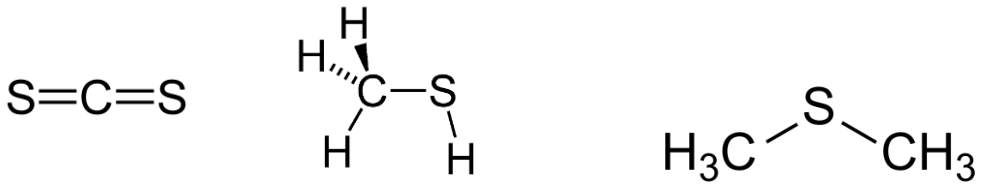


Fig. 5 Shows structural formula of CS_2 (left), Methanethiol (Mercaptomethane) (middle) and Dimethyl sulfide (right).

5.1.1 ROS and amino acid metabolism in relation to volatile sulfuric compound emission.

Mutations of genetic material because of oxidative damage is one of the first events involved in aging as well as the development of cancer. *Calenic et al. (2015)* suggest in their review about the interplay between oxidative stress and VOC production that different ROS may have different "affinities" for their target substrates. The oxidation of proteins has been studied by using different models involving proteins, peptides or amino acids which are exposed to ROS, such as superoxide and hydroxyl radicals formed after ionizing radiation. *Calenic et al. (2015*) state that ROS-mediated protein oxidation has a higher affinity for methionine and cysteine in an amino acid chain compared to other amino acids in the chain. Cysteine and methionine are sulfur containing amino acids that when broken down can give rise to a host of other VOCs. These new compounds can then initiate secondary reactions with each other to create newer compounds.

Glucose metabolism is accelerated in cancer cells, and there is an accumulation of glucose and methionine in cancerous cells, which can non-enzymatically react and form carcinogenic

substances. *Yamagushi et al. (2012*) writes that there has long been speculation regarding whether these reactions can produce sulfur-containing compounds in tumor tissue.

5.1.2 Hydrogen sulfide synthesis and its relation to CS2 and methanethiol production

Hydrogen sulfide (H_2S) stimulates vaso-relaxation, angiogenesis, and cellular bioenergetics according to *Szabo et al. (2013).* They state that H_2S can be synthesized endogenously from L-cysteine by cystathionine- β-synthase (CBS) and cystathionine-γ-lyase (CSE), and by the combined action of cysteine aminotransferase and 3-mercaptopyruvate sulfurtransferase (3-MST).) Transsulfuration, catalyzed by CBS, converts homocysteine to cystathionine, which cystathione gamma lyase converts to cysteine.

Szabo et al.'s study (2013) was done on the relationship between colon cancer and hydrogen sulfide. They found that the expression of CBS is significantly upregulated in human colonic adenocarcinomas. Also, they found that if you suppress the expression of CBS and CSE in colon cancer (cell line HCT116) by altering the genes coding for those enzymes with lentiviral vectors or by directly suppressing them with the CBS inhibitor amino-oxyacetic acid they observed both reductions in tumor cell proliferation and H_2S production. *Szabo et al (2013)* concluded that there could be a possible link between H_2S and colon cancer proliferation.

H_2S has also been shown to be produced by anaerobic bacteria in the colon *(Suarez et al 1997 and Picton et al 2007). Ramasamy et al. (2006)* also reported that increased levels of H_2S in the human colon may be a contributory factor in the development of colorectal cancer, while *Yamagishi et al (2012)* found increased H_2S in colon cancer and postulates that the increased concentrations of H_2S in cancer patients may be due to the production by cancer cells, or may be due to alterations in gut microbiota in response to cancer. *Kumar et al (2014)* found that H_2S is increased in gastric cancerous tissue. The H_2S seemed to be correlated with increasing pH in the stomach of the cancer patients as compared to the non-cancerous patients.

Whether from endogenous origin or from microbial origin, H_2S could have an important factor to play in the chemical interplay leading to CS_2 formation as will be explained further.

5.1.3 Volatile production from cysteine

Ahn et al (2016) shows in his study on radiation physics and chemistry that if you have increased concentrations of H_2S, it can react with CO_2 to produce carbonyl sulfide (COS) and water (H_2O). COS can then further react with H_2S to produce carbon disulfide (CS_2) and H_2O giving a potential origin for the increased CS_2 found in the cancerous tissue found in our study.

$H_2S + CO_2 \rightarrow COS + H_2O$ (Reaction 1)

$COS + H_2S \rightarrow CS_2 + H_2O$ (Reaction 2)

Ahn et al (2016) also found increases in the following VOCs in their study:

Amino acid	Volatiles	0 kGy	5 kGy	SEM
		——————Total ion counts × 10^4——————		
Cysteine	Mercaptomethane	0^b	4684^a	661
	Carbon disulfide	0^b	17841^a	1108
	Dimethyl disulfide	0^b	3555^a	656
Cystine	Acetaldehyde	0^b	62647^a	7742
	2-propanone	0^b	4723^a	284
	Carbon disulfide	102^b	472^a	43
	3-methyl pentane	796^a	89^b	125
	Hexane	35484^a	4087^b	4795
	Methyl cyclopentane	6671^a	2110^b	879
Methionine	Mercaptomethane	0^b	5198^a	1335
	2-Propenal	0^b	3757^a	846
	2-Propanone	0^b	655^a	38
	Dimethyl sulfide	0^b	120056^a	899
	Hexane	222^b	689^a	39
	Butanal	0^b	164^a	13
	(methylthio)-ethane	0^b	1510^a	67
	Thiophene	0^b	74^a	3
	3-(methylthio)-1-propene	0^b	230^a	8
	Dimethyl disulfide	131^b	208770^a	990
	Methyl ethyl disulfide	0^b	417^a	22

^{a,b} Means with no common superscript differ significantly ($P < 0.05$), $n = 4$.

Table 3 *Shows production of volatile compounds from sulfur-containing amino acid monomers solution by irradiation. Taken from Ahn et al. (2016) Radiation Physics and Chemistry 119 page. 82*

In their study, CS_2 was found to be increased after radiation of both cysteine and cystine. Two other disulfides; dimethyl disulfide and methyl ethyl disulfide were produced. The production of these substances is assumed to be due to:

1. Direct cleavage of the individual bonds in the amino acid chain
2. Secondary reactions from the byproduct of the cleaved amino acid with surrounding environment.

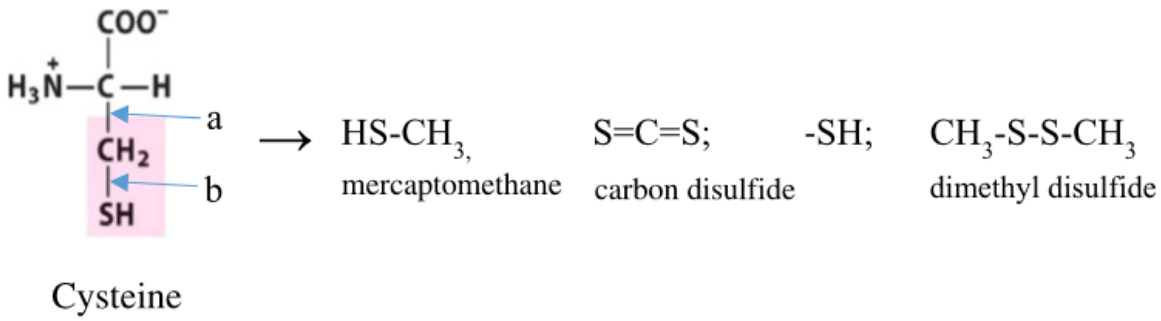


Fig. 6 Shows potential origins for methanethiol (mercaptomethane), carbon disulfide and dimethyl disulfide, depending on where you break the amino acid bond. Taken from Ahn et al. (2016) Radiation Physics and Chemistry page. 82.

By directly cleaving cysteine by irradiation, you can get methanethiol (mercaptomethane (HS-CH3)) by cleaving it at point (a). It is stated that dimethyl disulfide can then be produced by adding two methanethiol molecules together.

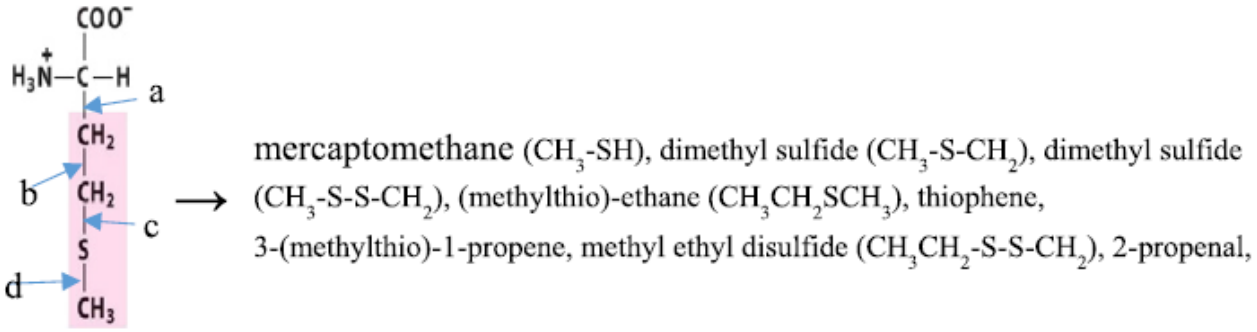
$HS\text{-}CH_3 + HS\text{-}CH_3 \rightarrow CH_3\text{-}S\text{-}S\text{-}CH_3 + 2H^+$ (Reaction 3)

If you cleave cysteine at point (b.) you will get SH^- which can react with another H^+ to get H_2S. H_2S can then result in increased CS_2 production as shown by reaction 1.

Shu et al. (1985) studied the thermal degradation of cysteine in water at pH 5.5 and pH 2.3, respectively, and reported that sulfur compounds were generated more readily at lower pH values as compared to higher pH values. The tissue samples in this study were all taken from the gastric mucosa.

5.1.4 Volatile production from methionine

In *Ahn et al.'s (2016)* study irradiation of methionine produced several other VOCs, including methanethiol (CH_3-SH), 2-Propenal (C_2H_3CHO), 2-Propanone (CH_3CH_2CO), dimethyl disulfide (CH_3-S-S-CH_3), butanal ($CH_3CH_2CH_2CHO$), methylthio-ethane ($CH_3CH_2SCH_3$), thiopene (C_4H_4S), 3-methylthio-1-propene and methyl ethyl disulfide (CH_3CH_2-S-S-CH_2), most of which were sulfur containing compounds. The amount of sulfur containing VOCs generated from methionine were more than 15-fold compared to that of cysteine.



Methionine

Fig. 7 Shows the potential origin of various VOCs (including methanethiol (mercaptomethane) and dimethyl sulfide) from irradiation of methionine. Taken from Ahn et al. (2016) Radiation Physics and Chemistry page. 83.

If you cleave methionine at point (c) or both (b) and (d) it will generate CH_2-S, which can then react with a H^+ ion to form methanethiol. Dimethyl sulfide (CH_3-S-CH_2) is produced if you directly cleave methionine at point (b). Dimethyl disulfide can be produced by adding two methanethiol molecules together (reaction 3). Methylthio-ethane (CH_3-CH_2-S-CH_3) is produced from the direct cleavage at point (a). The remaining compounds (thiophene, 3-(methylthio)-1-propene and methyl ethyl disulfide) was thought to be formed through multi-step interactions of irradiation products after the primary irradiation products were formed. These compounds were found in far less quantity than the other sulfur compounds.

Ahn et al.'s (2016) study shows that methionine, cysteine and cystine can be a source of carbon disulfide, dimethyl sulfide and methanethiol, as well as several other organosulfides. Other studies related to VOC's and cancer have found different variations of organosulfuric compounds increased from cancerous tissue *(Mochalski et al 2013, Rudnicka et al 2014). Ahn*

et al. (2016) shows that these variations can come from the same source by cross-reacting with each other, or by reacting with other compounds in their environment. This can be one explanation to why several studies on VOCs often find increases in different variations of VOCs, even when studying the same cell line or tissue. However, for this mechanism to be applicable in the context of gastric cancer one must assume that there are some alterations in the metabolic pathways of these amino acids in the cancer cells, or that the amino acid bonds can be cleaved by (for example) high levels of free radicals found in cancerous cells. *Calenic et al. (2015)* stated that methionine and cysteine had the highest affinity for ROS damage compared to the other amino acids.

5.1.5 Dimethyl sulfide and methanethiol production from methionine catabolism

Two main routes for methionine catabolism include the transmethylation and transsulfuration pathways. However, methionine is also shunted down the transamination pathway. This pathway involves an initial conversion of methionine to α-keto-ϒ-methylthiobuturate (KMTB). KMTB is then oxidatively carboxylated to 3-methylthiopropionate (MTP). This reaction is catalyzed by the 2-oxo acid dehydrogenase complex. From this point MTP can be metabolized further into the gaseous methanethiol. Methanethiol can then be broken down into simpler molecules such as H_2S, dimethyl sulphide and formic acid, with the end products being carbon dioxide and sulfur dioxide. *(Tavares et al 2016)*

5.1.6 Release of methanethiol, dimethyl sulfide and CS_2 from microbes.

Filpak et al (2012) found that sulfur containing volatiles were also abundantly released from *Streptococcus pneumonia (S. Pneumonia) and Hemophilus influenza (H. Influenza).* Methanethiol, dimethylsulfide, dimethyldisulfide, dimethyltrisulfide and CS_2 were the ones found to be significantly elevated. Volatile sulfur compounds (VSCs) were released abundantly by both species, comprising methanethiol, dimethylsulfide, dimethyldisulfide, dimethyltrisulfide and CS_2. *S. pneumoniae* also released one unique VSC 3-(methylthio)propanal, while *H. influenzae* released three specific VSCs, namely ethyl methyl sulfide, S-methyl thioacetate and 3-(ethylthio)propanal. Methanethiol and dimethylsulfide has also been found to be released from *Pseudomonas aeruginosa* and *Staphylococcus aureus* according to *Filipak et al. (2012*

5.1.7 VOC production from septa used for GC-MS analysis

Ulanovska et al. (2012) found increased CS_2 production in some septa used by the GC-MS analysis. The increases were found in brands consisting of butyl + polytetrafluoroethylene and one brand consisting of butyl + rubber.

5.2 2-Pentanone and 2-Butanone, 3-methyl-

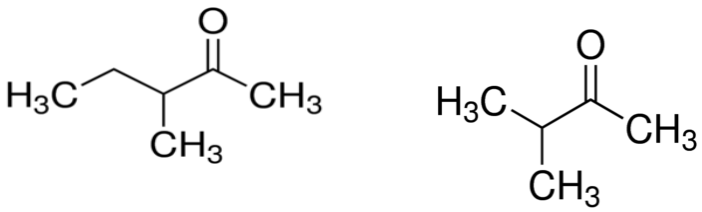


Fig. 8 Shows the structural formula of 2-pentanone (left) and 2-Butanone, 3-methyl (right)

2-Pentanone and 3-methyl-2-butanoone are both ketones. They are isomers, meaning they have the same chemical formula C5H10O, but different structural arrangements. Evidence seems to suggest that ketones could be formed through fatty acid oxidation, oxidation of secondary alcohols or oxidative stress reactions of lipid structures within the cell. Several studies, especially with regards to lung cancer, have found increased 2-butanone and 3-hydroxy-2-butanone. However, no studies were found relating 3-methyl-2-butanone to cancer. 2-Pentanone, 2-butanone and 3-methyl-2-butanone have all been found to be emitted from P. aeruginosa, which is one of the bacteria species that can inhabit the harsh stomach environment *(Shestivska et al. (2012)*.

5.2.1 Formation of ketones through lipid metabolism

Ketones can be produced from various chemical processes. Several ketones have been found in several cancer studies in relation to VOCs. Among them acetone and β-hydroxybutyrate have been described as potential biomarkers of oesophago-gastric cancer. β-Hydroxybutyrate has been shown to be up-regulated in the serum of patients with gastric cancer using GC–MS and in the serum of patients with oesophageal cancer using nuclear magnetic resonance spectroscopy (NMR). *(Abassi-Ghadi et al. 2013)*

Lipid metabolism is believed to be the main mechanisms for endogenous ketone production. One well known example is the conversion of acetyl CoA derived from fatty acid oxidation into ketone bodies by the liver (and kidneys to a lesser extent). Ketone bodies are an important secondary source of energy to the peripheral tissues because:

1) they are soluble in aqueous solution and do not need to be incorporated into lipoproteins or transported by albumin to get to the target cells.

2) they are produced in the liver during periods when the amount of acetyl CoA present exceeds the oxidative capacity of the liver, and

3) they are used by extrahepatic tissues, such as the skeletal and cardiac muscle, renal cortex and brain. Ketones thus spare the body of glucose, which is important during prolonged periods of fasting

In the peripheral tissues, they can be reconverted to acetyl CoA, which can be oxidized by the TCA cycle *(Harvey et al. 2011 p. 195-196)*

Fasting has been shown to increase the concentrations of 2-pentanone along with acetone and 2-butanone. *(Velde et al. 2007).* The exact origin and mechanism of 2-pentanone remains unclear, but it is assumed to arise through β-oxidation. *(Walker et al. 2014)* In a study done on seven fasting monks, the acetone concentrations after fasting (4.1 ppmv (mol/mol)) were even very close to the range of diabetic breath (1.7–3.7 ppmv). *(Statheropoulos et al 2006)* While fasting, the liver is flooded with fatty acids released from fat tissue. The elevated hepatic acetyl CoA, which is primarily made by fatty acid degradation, inhibits pyruvate dehydrogenase and activates pyruvate carboxylase. Pyruvate is carboxylated by pyruvate carboxylase to form oxaloacetate (OAA). The OAA produced is used by the liver for gluconeogenesis instead of the TCA cycle, while acetyl-CoA is used for ketone body

synthesis. This channels acetyl CoA away from gluconeogenesis and into ketogenesis. *(Harvey et al. 2011 p.195)*

Walker et al (2014) also state in their study on 2-pentanone production from hexanoic acid by penicillin roqueforti, that from the current evidence, methyl-ketones seem to be formed by cycles of incomplete β-oxidation of fatty acids.

"It is proposed that β-oxidation proceeds normally to the formation of medium-chain 3-oxoacyl-CoA intermediates but is then halted at the medium-chain acyl-CoA thiolase reaction. Thioesterase(s) release Coenzyme A (CoASH) from the accumulating intermediates and the 3-oxoacids are decarboxylated to methylketones. CoASH might then be recycled to initiate oxidation of more fatty acids, but their metabolism would again be held up at the thiolase bottleneck". (Walker et al 2014 p.2)

This is also supported by *Lewis et al (1970), Kinsella et al (1976), Baltazar et al (1999) and Fadda et al (2002)*

Walker et al. (2014) came up with a hypothesis that 2-pentanone could be formed from hexanoic acid via the following mechanism:

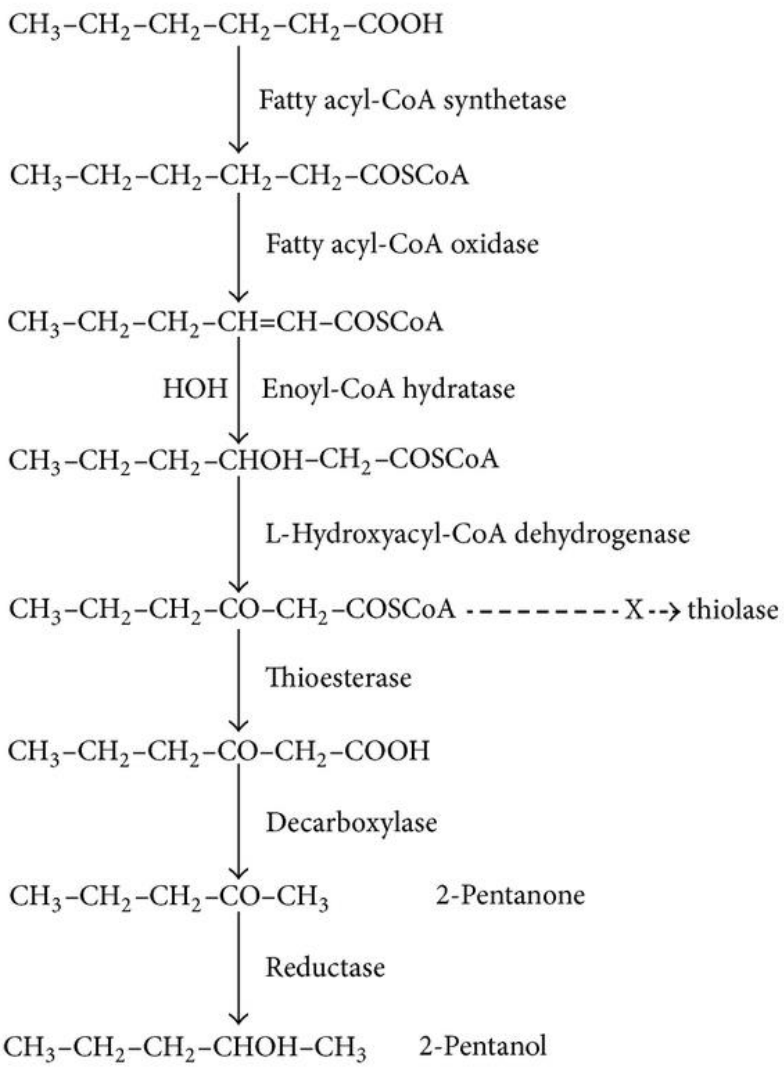


Fig. 9 Shows proposed mechanism how 2-Pentanone could be formed from hexanoic acid. Taken from Walker et al 2014 p.2.

Walker et al (2014) confirmed their hypothesis by deuterating the C2 and C3 carbons of hexanoic acid. They provided the following mechanism:

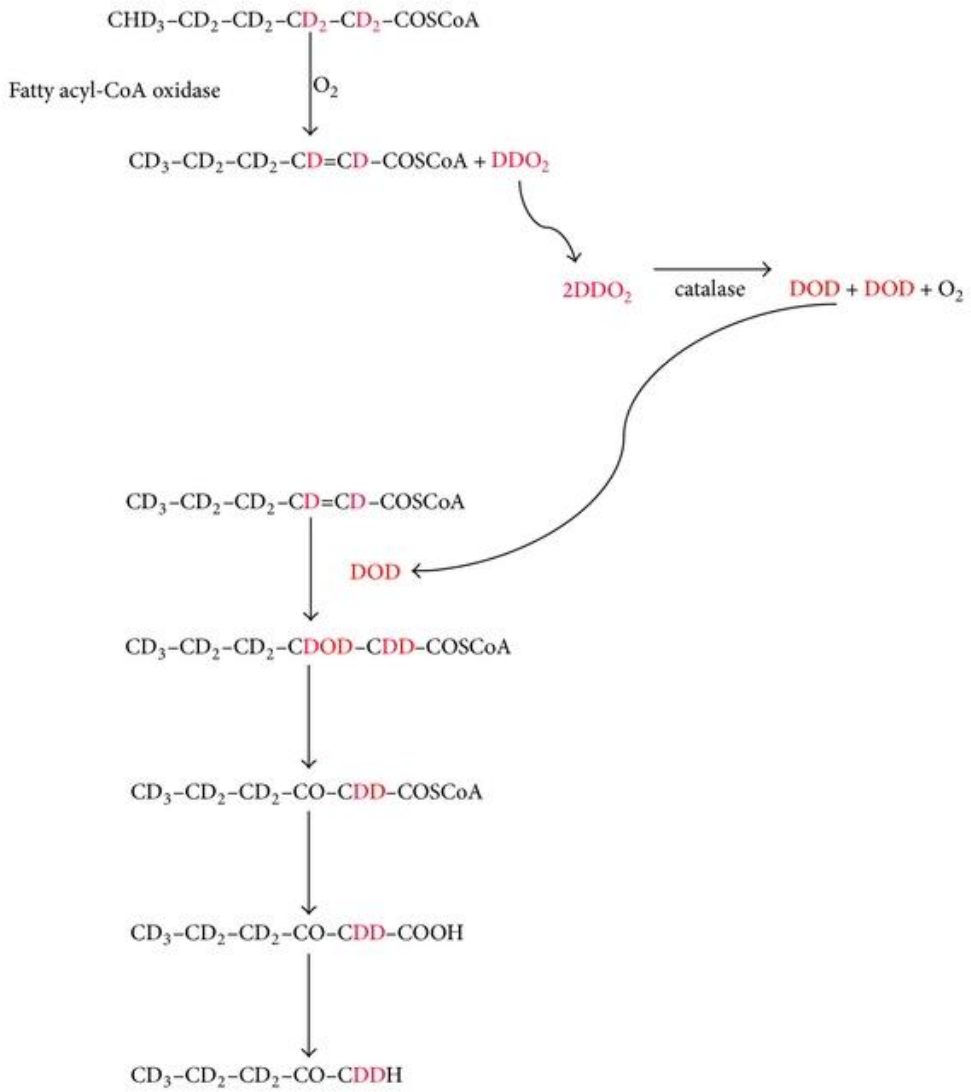


Fig. 10 Shows the proposed pathway for producing 2-pentanone from deuterated hexanoic acid. The "D" indicates the deuterated carbons. Taken from Walker et al. 2014 p.9.

Walker et al. (2014) also proposes that it is quite likely that the same mechanism is one that could be used by humans. The reasoning behind this is that:

1. Excretion of 2-pentanone is increased in fasting ketosis when large amounts of fatty acids released from adipose tissue are delivered to the liver.

2. Fatty acid oxidation is known to be increased by fasting.
3. The adaptive response to increased β-oxidation is mediated by the transcription factor peroxisome proliferator-activated receptor alpha (PPAR-α), which is induced by glucocorticoids and activated by fatty acids and fatty acyl-CoAs.
4. The activation of PPAR-α increases the expression of all the enzymes of the mitochondrial and peroxisomal β-oxidation pathways. *(Walker et al 2014)*
5. 2-pentanone is decreased rapidly in response to glucose administration *(Walker et al 2008)*

They argue that 2-pentanone could be derived from either peroxisomal or mitochondrial β-oxidation. In favor of peroxisomes are firstly that peroxisomal β-oxidation does not proceed to completion, but stops after oxidation of C6 fatty acids leading to increased six-carbon derivatives such as hexanoic acid. Also, fasting ketosis is often accompanied by excretion of medium-chain dicarboxylic acids from fatty acid catabolism, which are only produced by β-oxidation from peroxisomes. *(Walker et al. 2014)*

Against peroxisomal β-oxidation is that 2-pentanone production requires oxidation of hexanoyl-CoA, which is right at the low-end of specificity for of the mammalian straight-chain peroxisomal acyl-CoA oxidase (ACOX1). In addition, peroxisomal -oxidation does not have a significant role in energy production *(Walker et al. 2014).*

The idea that ketones are produced from the oxidation of fatty acids seem to be supported by many studies. In a recent review by Lubes et al (2017), they found that acetone, 2-butanone and 2-pentanone seems to be the ones found most frequently in association with cancer. In *Hanai et al's (2012)* study, they measured VOC production in both the headspace of cultured cells of the A549 cell line (adenocarcinoma), as well as in the urine of mice with lung adenocarcinoma. They found significant increases in several ketones including 2-pentanone, 2-butanone and acetophenone.

5.2.2 Formation from secondary alcohols

From a chemical perspective, we know that ketones can be formed from oxidation of secondary alcohols. Primary alcohols are oxidized into aldehydes. In human's alcohols are mainly oxidized by the enzyme alcohol dehydrogenase (ADH) and possibly by the CYP-450

system. ADH is found in several tissues, but in largest quantities in the liver and stomach lining.

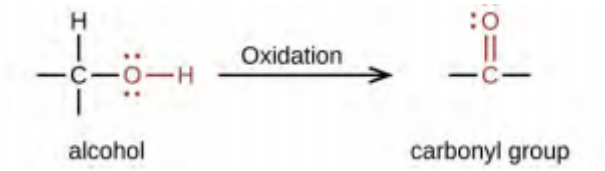


Fig. 11 Oxidation of secondary alcohols produces ketones.

The exact metabolic source of secondary alcohols in humans is still not clear. However, *Mochalski et al. (2015)* state that there is already evidence for the conversion of secondary alcohols into ketones by ADH. Although ADH has a higher affinity for primary alcohols, they also oxidize secondary alcohols. This is also supported by a study done by *Plapp et al (2015)* on ADH in rats. They found that ADH in rats metabolized both primary and secondary alcohols by oxidizing them into ketones.

Several studies done concerning liver cancer and other liver diseases with relation to VOCs have found increases in various ketones. *(Van den Velde et al. (2008), Fernandez del Rio et al. (2015), Mochalski et al. (2015))* In *Van den Velde et al's (2008)* study done on patients with liver cirrhosis they found significant increases in acetone, 2-pentanone and 2-butanone. Dimethyl sulfide was also found which is thought to be an important metabolite for the odor in fetor hepaticus. The increased concentration of ketones in the case of cirrhosis was believed to be due to hepatic insulin resistance due to liver steatosis and end stage liver failure. Insulin resistance leads to increased triglycerides and free fatty acids. The fats are believed to be oxidized into ketones via the lipolysis pathways.

Another study done on rats found that if they inhibited the CYP2E1 enzyme of the cytochrome P450 enzymes in the liver, there was significant increases in the same compounds. *(Mathews et al 1997)* It has also been observed that the levels of CYP enzymes are decreased in patients with liver cirrhosis supporting this finding. *(Fernandez del Rio et al (2015)*

5.2.3 Presence in healthy subjects with halitosis

2-Penanone was also increased in Velde et al's study (2006) on healthy subjects with halitosis.

5.2.4 Formation from microbes

A study done on trying to differentiate various lactobacilli from each other by differences in volatiles, found that several strains of lactobacilli all produced various ketones, which allowed them to be differentiated from another. 2-butanone, 2-pentanone, 2-heptanone and 3-methyl-1-butanol were the ones that was significantly increased. In their study, they used GC-MS and PCR to discriminate between species of *Lactobacillus casei, Lactobacillus paracasei* and *Lactococcus lactis*. The proposed mechanism for the ketone production was through β-oxidation of fatty acids. (Gallegos et al 2017)

Filipak et al's study (2012'9 on VOC release from *S. Pneumonia and H. influenza* found that both bacteria emitted 2-pentanone, but at trace levels. They measured around 0,4 p.p.b for both species.

Tait et al. (2014) found increased 2-pentanone and 2-butanone released from *E. coli*, while *Shestivska et al. (2012)* found increased emissions of 2-pentanone, 2-butanone and 3-methyl-2-butanone.

5.3 Pyridine

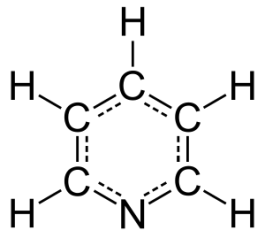


Fig. 12 Shows structural formula of pyridine.

The mechanism between pyridine emission and cancer is still unclear. It is, however, considered a VOC marker for tobacco exposure. *(Kapishon et al 2013)*

Pyridine has been considered a potential diagnostic compound for use in diagnosing etiology or pathogenesis of periodontal disease. In a study done by *Kostelc et al (1981)* they found increased pyrimidine and methylpyridines in the saliva of patients suffering from periodontitis by using GC-MS. More interestingly, these compounds were virtually absent when comparing them to the healthy control group. This lead to the conclusion that these compounds could be related to the disease process.

Emission of pyridine has been found in lung cancer (Filipak et al. 2014), but no studies were found linking it to gastric cancer. Any cellular metabolic pathway that leads to pyridine emission is still unclear.

5.4 Isoprene

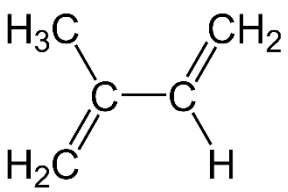


Fig. 13 Shows structural formula of isoprene.

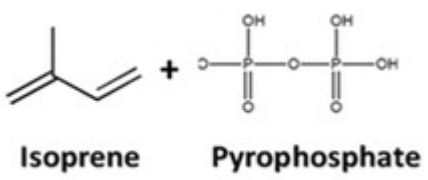
Isoprene can be formed endogenously, and is connected to the mevalonate pathway (MVP). The endogenous production rate of isoprene has been calculated to around 0.15 $\frac{\mu mol}{kg} per\ hr$. Concetrations in the blood range between 15 to 70 nmol/L. Isoprene is also found in human breath at a concentration range of 10-30 nmol/L. (IARC 1994) The endogenous synthesis via the MVP is further supported by the observation that there is a decrease in isoprene and sterol production after the administration of statins. Statins are a group of lipid-lowering drugs that block HMG-CoA-reductase, one of the first enzymes involved in the MVP. The decreased isoprene could be measured in both the breath and plasma of the patients. Additionally, a high cholesterol diet reduces the isoprene levels in exhaled breath. A high cholesterol diet inhibits HMG-CoA via a negative feedback mechanism *(Stone et al. 1993, Quinsley et al. 1971).* The breath and plasma levels of isoprene is thus highly correlated with cholesterol levels and the MVP.

5.4.1 The mevalonate pathway.

The mevalonate pathway is an essential pathway used by both eukaryotes and prokaryotes. It is most commonly known for its use in cholesterol synthesis, however, it is now known to have a much broader scope of functions as will be discussed shortly. The MVP is now known to be involved in several cells signaling pathways and thus plays an important role in cell proliferation and growth. Several cancer studies are now considering the MVP and its role in tumorigenesis and potential therapeutic options in cancer. (Likus et al. (2016), Yeganeh et al. (2014), Karlic et al. (2015))

The first step in the mevalonic pathway is the formation of 3-isopentenyl pyrophosphate (IPP) from acetyl-CoA. This is a multi-step process. IPP is in essence an isoprene molecule + pyrophosphate.

IPP=



The MVP starts with the formation of 2-Hydroxy-3-methylglutaryl COA (HMG-CoA) by adding acetyl-CoA and acetoacetyl CoA. In the mitochondria HMG-CoA is converted into acetyl CoA and acetoacetate, while in the cytosol it is converted to Mevalonate via the reaction:

HMG-CoA + 2NADPH + 2H+ → Mevalonate + $2NADP^+$ + CoA

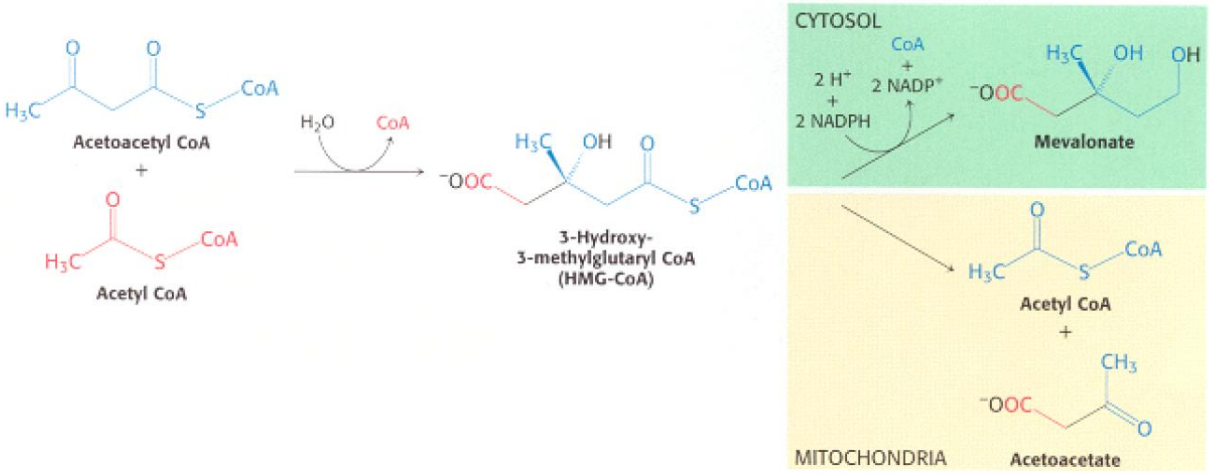


Fig. 14 Shows the different conversions of HMG-CoA in cytosol an mitochondria. Taken from Berg et al. 2012. Biochemistry 7th ed. Page 796.

Mevalonate is then converted to IPP via three consecutive reactions, all of which use one molecule of ATP, followed by a decarboxylation in the last step.

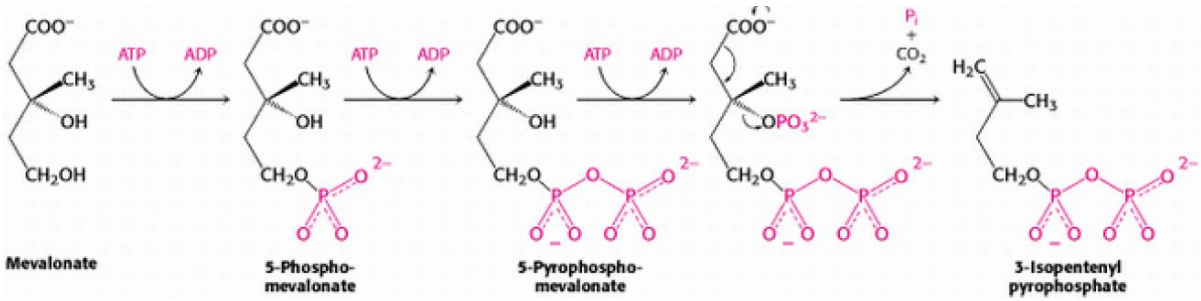


Fig 15 Shows 3 step process from mevalonate to IPP. Taken from Berg et al. 2012. Biochemistry 7th ed. Page 796.

IPP can then be isomerized in to Dimethylallyl pyrophosphate (DMAPP)

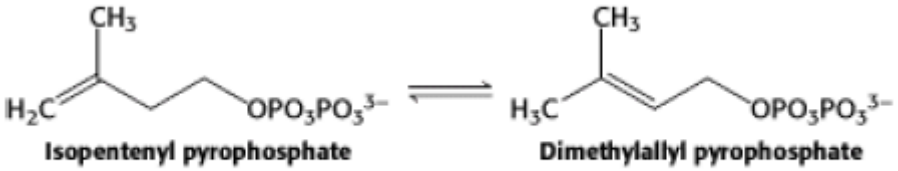


Fig. 16 Shows the isomerization of IPP to DMAPP. Figure taken from Berg et al. (2012). Biochemistry 7th ed. Page 79

IPP and DMAPP can then condense with each other, creating a 10-chain carbon molecule called geranyl pyrophosphate (GPP). This can then react with another IPP forming a 15-carbon chain called farnesyl pyrophosphate (FPP). Two FPP molecules can then condense to for a 30-chain carbon, squalene. The formation of squalene is the pathway used to for cholesterol and all steroid molecules. *(Berg et al. 2012)* In mammals, the synthesis of GPP and FPP is catalyzed by GPP synthase and FPP synthase, respectively *(Coppens 2013)*.

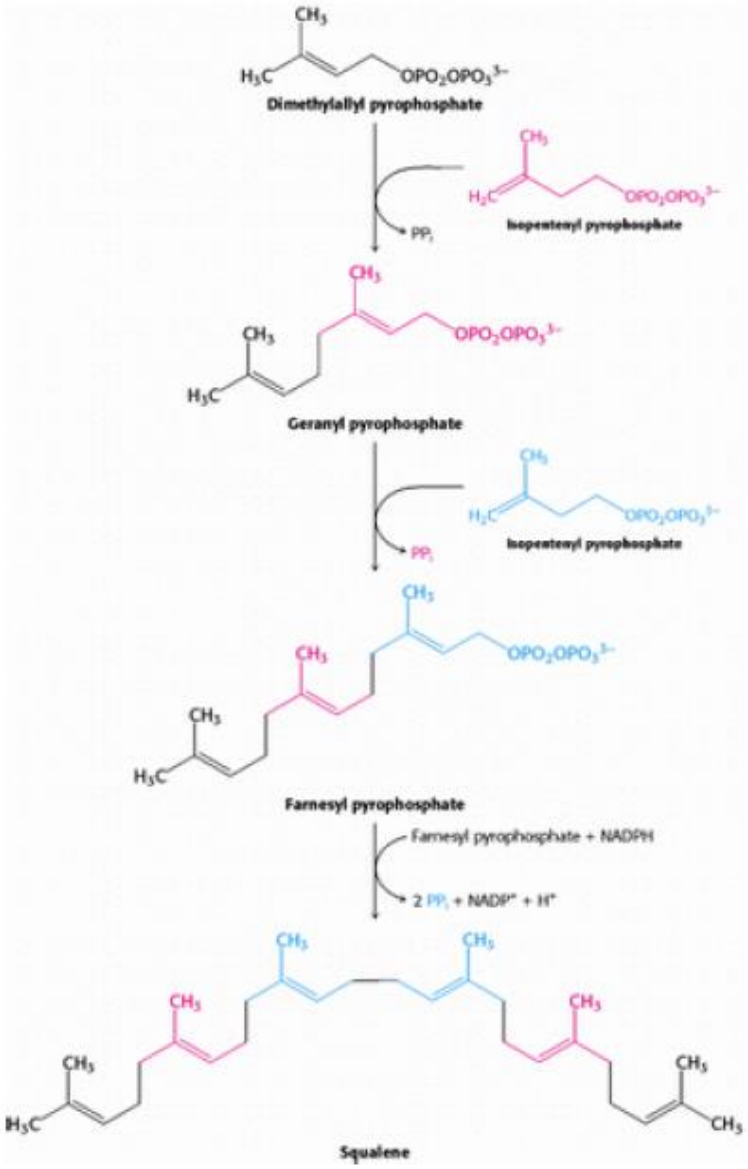


Fig. 17 Shows the condensation of IPP and DMAPP into GPP, which is further condensed to FPP and squalene. Taken from Berg at al. 2012. Biochemistry 7th ed. Page 797

5.4.2 The MVP and cancer research

The MVP is now known to have several more functions than just cholesterol synthesis. It is involved in the production of several bioactive intermediaries and end-products. After the formation of FPP the MVP can diverge into numerous other pathways including cholesterol biosynthesis, ubiqionone formation, formation of Heme A prosthetic group, dolichols and isoprenoids *(Moutinho et al. 2017).* Figure 17 shows a schematic diagram of sterol and non-

sterol pathways that can follow the MVP. Isoprenoids are used for prenylation. The main isoprenoids are FPP and geranylgeranyl pyrophosphate (GGPP), which is formed by adding another IPP to FPP and regulated by GGPP synthase. Prenylation is the process of bridging a cytosolic protein molecule to a lipid membrane. These proteins are known to include several members of the Ras family, which is made up of various small GTP-binding proteins. Ras proteins are involved in extremely complex cell signal transduction pathways; thus, they are implicated in the regulation of cell growth, differentiation and survival. (Likus et al. (2016) Two examples of such proteins include Rho, which is a protein involved in the termination of transcription, and Rab which are GTPases involved in membrane trafficking such as vesicle formation, membrane fusion and vesicle movement along the cytoskeleton. Eukaryotic cells have three different enzymes regulating prenylation; farnesyl transferase (FTase) and geraylgeranyl transferase I and II (GGTase I/II) *(Mauer-Stroh and Eisenhaber (2005))* It is through prenylation of proteins that the MVP is believed to have the potential to impact cell function to a very large extent. Dolichols are long chain organic carbon compounds that are made of a varied number of isoprene units that terminate in an α-saturated isoprenoid group, containing an alcohol functional group. Dolichols play a large role in N-glycosylation (the anchoring of carbohydrate molecules to lipid membranes).

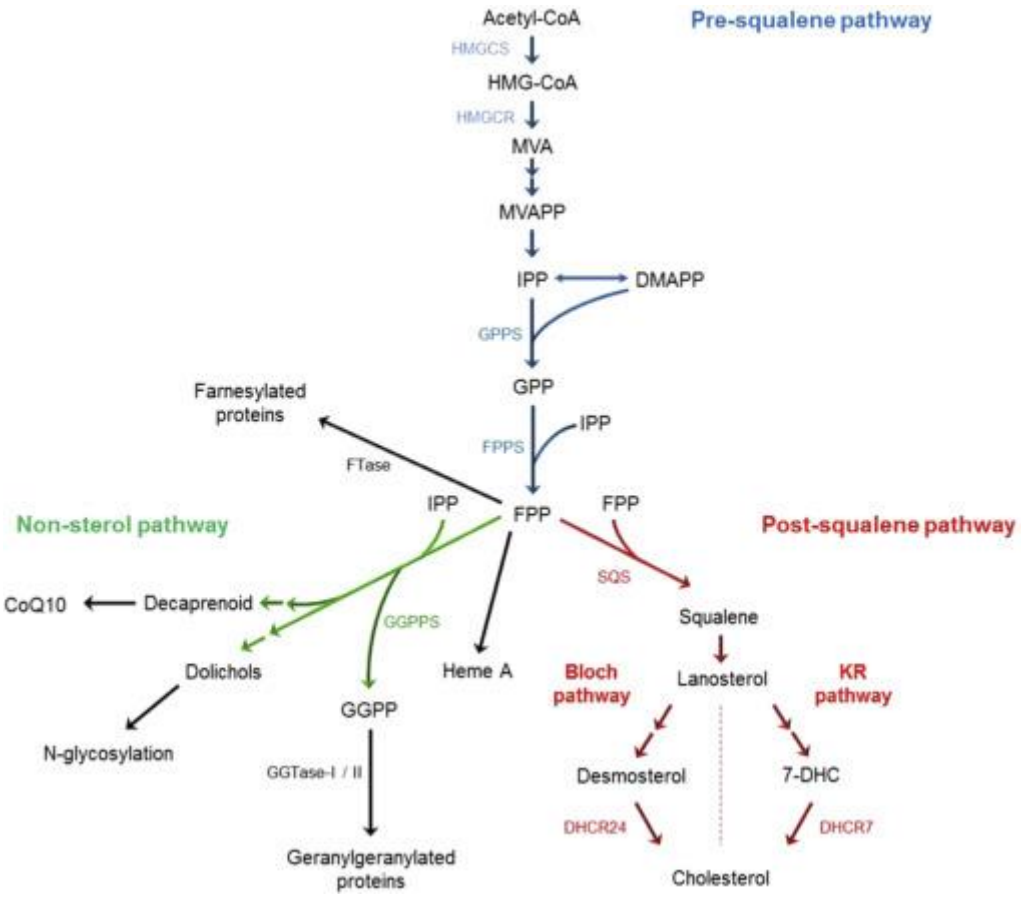


Fig. 18 Schematic showing possible outcomes through the MVP. Taken from Moutinho et al. (2017), The mevalonate pathway in neurons; It's not just about cholesterol. Page 2.

Mutilations in the Ras oncogenes is one of the most common oncogene found to be mutated in human cancer and are found in 20-25% of all human tumors (Downward (2003)). The Ras oncogene mutations are usually point mutations on one of the three genes (most commonly KRAS), that renders the GTP switch in the Ras protein non-functional. This then results in a continuously activated protein. Disruption of prenylation will result in Ras proteins being contained in the cytosol and inactive as they are not anchored to any lipid membrane *(Jackson et al. (1990).*

Several studies have shown that cells that are cholesterol depleted fail to enter the S-phase, and are thus halted in either the G1 or G2 phases *(Keyomarsi et al. 1991, Singh et al (2013), Fernandez et al. (2004)).* HMG-CoA reductase (HMGR) is the rate limiting enzyme in the MVP, and it is therefore the most regulated enzyme. It is regulated by direct inhibition, regulation of enzyme production via transcription, translation, phosphorylation and degradation, thus adjusting the activity in response to a range of stimuli. *(Burg and*

Espenshade (2011)). The use of HMGR reductase inhibitors for inhibition of tumor growth seems to be somewhat controversial still *(Lubik et al. 2016).*

In this study isoprene levels were decreased in the cancerous tissue, which could be suggest that the MVP is not used in extent that it is in the healthy tissue. This also correlates with the reduced ability for cell regulation that is provided through the MVP.

5.4.3 Isoprene and VOC research

The origin of endogenous isoprene seems to be established, however, the mechanism of which VOC concentrations of isoprene is different in cancer patients compared to healthy controls is still somewhat unclear. It seems to be considered one of the most important biomarkers used in diagnosing lung cancer *(Sakamura et al. 2017).*

Bajtarevic et al. (2009) found concentrations of isoprene, acetone and methanol of exhaled breath to be slightly lower ($p < 0,01$ for isoprene and acetone while $p < 0.011$ for methanol) in lung cancer patients when compared to healthy volunteers. *Rudnicka et al. (2011)* also compared the breath of 23 patients (17 males and 6 females) to 30 healthy volunteers. They found significantly increased levels of propan, CS_2, 2-propenal, ethyl benzene and isopropyl alcohol in the cancer subjects. The levels of isoprene, methanol and acetone was decreased when compared to the healthy group.

Sakamura et al. (2017) did a study where they analyzed exhaled breath in lung cancer patients and healthy subjects using GS-MS, and did a statistical analysis to see if they could diagnose lung cancer based on the combination of multiple lung cancer related VOCs. They managed to reduce the number of VOCs (63) down to five, namely isoprene, acetonitrile, CHN, methanol and 1-propanol, and use those to diagnose lung cancer with an 89% sensitivity. They stated that the most important markers for lung cancer diagnosis seems to be isoprene and acetonitrile.

Poli et al. (2005) did a study measured the breath concentrations of VOCs in exhaled air in lung cancer patients before, a month and three years after surgery. 12 VOCs were found to be elevated in the cancer group before surgery when compared to the healthy group. Those were 2-methylpentane, pentane, ethylbenzene, xylenes, trimethylbenzene, toulene, benzene, decane, octane and pentamethylhepta. The levels of isoprene were not statistically elevated in the cancer group. Interestingly, on the three years follow up study they found decreased levels of isoprene, benzene, pentane, toluene and ethyl benzene in their subjects *(Poli et al. 2008*)

Xu et al. (2013) found elevated levels of isoprene, 2-propenitrile, furfural, 2-butoxy-ethanol and 6-methyl-5-hepten-2-one in patients with gastric cancer and/or peptic ulcer disease.

5.5 Benzene

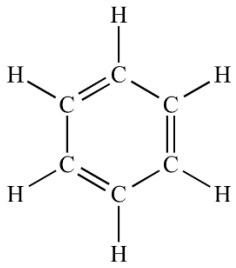


Fig. 19 Shows structural formula of Benzene.

Benzene is a well-known carcinogen and strongly linked to bone marrow failure. Large quantities of epidemiological data link benzene to diseases like aplastic anemia, acute and chronic leukemia, myelodysplastic syndrome as well as cardiovascular disease. *(Kasper et al. (2012), Bard et al. (2014)*

Benzene can be measured in urine, blood and breath of humans; however, benzene is also metabolized in the human body so these measurements have their limitations *(Agency for Toxic Substances and Disease Registry. (2007).* In humans, benzene is enzymatically converted to various oxidation products such as phenol, cathechol, hydroquinone, muconic acid, phenylmercpturic acid and 1,2,4-trihydroxybenzene. This is mainly believed to be done in the liver by CYP-p450 enzymes, quinine oxoreductase, glutathione and myeloperoxidase (MPO). Benzene is then broken down into several byproducts including polyphenols. In the bone marrow MPO convert these polyphenols to benzoquinones. It is these metabolites that induce the gene toxicity associated with cancer, and does so by several mechanisms including increasing oxidative stress, disruption of microtubules, alteration in DNA methylation and inhibition of topoisomerase II *(Snyder and Hedli (1996), Smith (2010).*

Aromatic compounds such as benzene, styrene, toluene, etc. are believed to come from exogenous sources such as pollution and cigarette smoke. *Hakim et al. (2012)* found increased

concentrations of benzene, toluene and 2,5-dimethyl furan in the breath of smokers versus non-smokers. *Ulanowska et al. (2011*) also found increased benzene, acetonitrile and furan derivatives in smokers.

Navaneethan et al. (2014) analyzed headspaces from bile samples, including from patients with pancreatic cancer and patient's benign biliary conditions. The concentrations of 6 compounds including benzene were increased in patients with pancreatic cancer compared with controls. *Mochalski et al. (2014)* analyzed VOCs in the blood and breath of chronic kidney disease patients. Excluding contaminants, six compounds (benzene amongst them) changed their blood and breath levels during the hemodialysis treatment. Benzene was also increased in *Poli et al's (2005 and 2008).* studies on lung cancer patients before and after resection. Benzene was found to be increased before resection and decreased 3 years after.

Zhu et al. (2010) reported emission of benzene from *H.pylori*. Why benzene was decreased in the cancerous tissue compared to the non-cancerous tissue is still unclear.

5.6 Gamma-Butyrolactone (GBL)

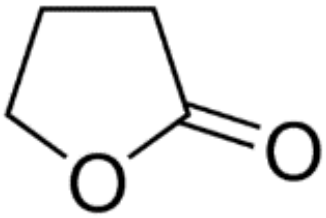


Fig. 20 Shows structural formula of gamma-butyrolactone.

5.6.1 Endogenous formation

GBL can be formed endogenously, and in combination with 1,4-butanediol they can form gamma-hydroxybutyrate (GHB). This is a rapid reaction catalyzed by lactonases *(Kapoor et al. (2013).* GHB is the precursor to the neurotransmitter gamma-aminobutyrate (GABA), which is a nervous system depressant and one of the main inhibitory neurotransmitters used by the central nervous system (CNS). GBL has been detected in blood and feces, as well as from adipose tissue and neurons *(The Human Metabolome Database (HMDB)).*

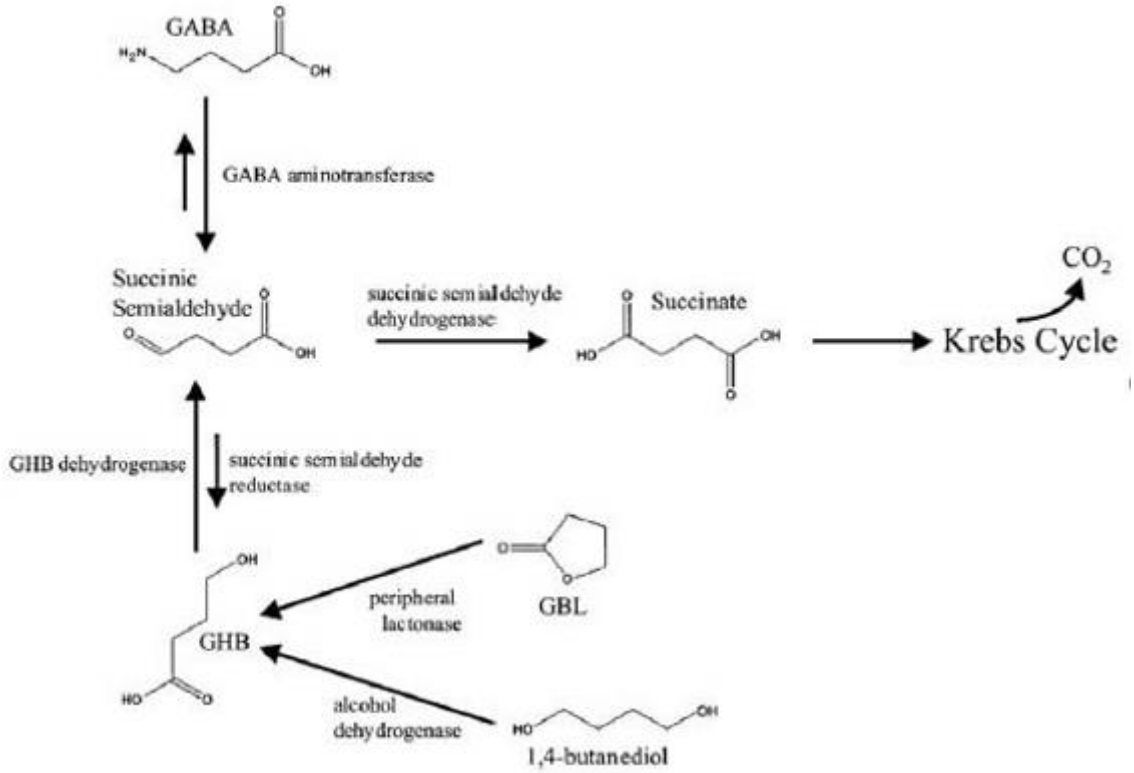


Fig. 21 Shows the formation of GBL and 1,4-butanediol into GHB, which can then be further converted to succinic semialdehyde and GABA. (Figure taken from Kapoor et al. GHB acid: a rage or reprieve (2013) page 3.)

GABA can also be metabolized to succinic semialdehyde which can either undergo conversion back to GHB via succinic aldehyde reductase, or go through the TCA-cycle to produce energy (*Kapoor et al. 2013).* GLB is believed to be formed endogenously from GABA, *(The Human Metabolome Database (HMBD))* and reversing the above mechanism could be one potential source of its endogenous production. However, no studies showing a link between VOC emission and cancer was found. *Rudnicka et al. (2011*) detected levels of butyrolactones in their cancer subjects, but it was deemed non-significant.

GBL was found to be increased using GC-MS in cells infected with herpes simplex virus type 1. *(Rochford et al. (2016)*

5.6.2 Exogenous formation

Both GHB and GBL are active metabolites from the anti-cancer drug group fluoropyrimidines (UFT). *(Nagai et al. 2008 and Emi et al. 2007)* The drugs include Fluorouracil (5-FU), Tegafur, Doxifluridine, Carmofur and Capecitabine.

Nagai et al. (2008) measured the serum GBL before and after UFT therapy in 22 cancer patients as well as 5 healthy volunteers. They found that the GBL levels after the UFT administration was significantly increased with a p value of <0.0001). They also concluded that GBL inhibits vascular endothelial growth factor (VEGF), thus inhibiting angiogenesis. This is believed to be one of the mechanisms in which UFTs inhibit tumor growth.

6. CONCLUSION

The results found in this study shows that the analysis of VOCs is a promising field of research into its potential in gastric cancer diagnosis. This study has identified increased emission levels of CS^2, 2-pentanone, 3-methyl-2-butanone and pyridine and decreased emission of isoprene, gamma-butyrolactone, benzene and dimethyl sulfide in gastric cancer tissue. The ketones, sulfuric compounds and isoprene have been found in several previous cancer studies, and hopefully this will add to the further understanding and potential discovery VOC biomarkers for gastric cancer. Currently VOC analysis for biomarkers in cancer screening is still in the early pre-clinical phase, and need more work before it can be used in practice.

One of the main goals of using VOCs in cancer detection is to develop a relatively easy and non-invasive method for screening. This could have a significant clinical impact as the majority of gastric cancer is diagnosed in the later stages of disease. The potential benefits of such a screening device could include:

- Earlier detection of gastric cancer, which is directly correlated to improved prognosis.
- Reduce costs of therapy in cancer patients
- Increase diagnostic rate of gastric cancer via population based screening

Screening could be done via several methods including exhaled breath, blood and urine analysis. The findings found in this study seems to indicate that gastric cancer cells have altered metabolic processes which can lead to differences in VOC formation when compared to normal cells. The exact origin of these VOCs is very complex, but due to the large differences in CS_2, 2-pentanone, 3-methyl-2-butanone and pyridine seems to indicate that they could be emitted from the cell. If the origin was microbial, from exogenous pollutants or environmental, you would expect more similarities in the profiles found.

If we consider CS_2 the differences in emission mass from cancerous to non-cancerous was so large that it cannot be denied that some process is altered in the cancerous cells. The median mass for non-cancerous tissue was 1.4 pmol, while for the cancerous tissue 19.9 pmol, which is around 14 times greater mass emitted from the cancerous tissue. The highest upper range of non-cancerous was 21 pmol compared to 260 pmol for cancerous. By using CS_2 as an example it is very evident that something is very different in the diseased tissue. VOC profiles do not have to be diagnostic of disease to be useful. One suggestion that could be interesting

for future studies on VOCs is to do large group studies on healthy individuals to try and determine a “range of normal”, similar to what we currently have for blood analysis. Screening VOCs using a simple non-invasive method, such as a breath test, could then give an indicator of abnormal VOC profiles, which could warrant further investigation. At the moment VOC analysis is still relatively cumbersome and time consuming and not necessarily ready for routine clinical practice. Newer and easier VOC analysis equipment is needed in order for day to day screening in clinics and hospital. However, by researching the potential that VOCs have in disease will likely lead to its development.

7. AKNOWLEDGEMENTS

My most heart-felt thanks go out to my family and friends. It is only through their emotional and financial support that I have been able to complete my medical degree.

I am sincerely grateful for the help and guidance provided to me by Prof. Mārcis Leja and Dr. Pawel Mochalski though this whole process.

My gratitude also goes to the Riga East University Hospital and University of Innsbruck for their help in providing the surgical samples and for the use of the GC-MS analysis.

DISSEMINATION OF PUBLICATIONS

- This work is part of a publication that is currently in the submission phase

- The authors contribution to this publication focused on VOC research and analysis of potential origins and metabolic pathways. Parts of this research has already been used in a presentation during the United European Gastroenterology week in Barcelona, 2017.

- The abstract will be submitted to the International Medical Conference, University of Latvia.

8. BIBLIOGRAPHY

1. Abbassi-Ghadi, N., Kumar, S., Huang, J., Goldin, R., Takats Z. and Hanna G.B. Metabolomic profiling of oesophago-gastric cancer: A systematic review. European Journal of Cancer (2013)

2. Agency for Toxic Substances and Disease Registry. U.S. Department of Health and Human Services. Public Health Service Agency for Toxic Substances and Disease Registry. (2007).

3. Ahn, D.U, Lee, E.J, Feng, X., Zhang, W., Lee, H.J, Jo, C. and Nam K. Mechanisms of volatile production from sulfur containing amino acids by irradiation. Radiation physics and chemistry 119. 80-84. (2016)

4. Altomare, D.F., Di Lena, M., Porcelli, F., Trizio, L., Travaglio, E., Tutino, M., Dragonieri, S., Memeo, V., and De Gennaro, G. Exhaled volatile organic compounds identify patients with colorectal cancer. British Journal of Surgery 100: 151 (2013)

5. Amieva, M. and Peek, R.M. Pathology of Helicobacter Pylori-Induced Gastric Cancer. Gastroenterology. Vol. 150 pp. 64-78. (2016)

6. Bajtarevic, A., Ager, C., Pienz, M., Klieber, M., Schwarz, K., Ligor, M., Ligor, T., Filipak, W., Denz, H., Fiegl, M., Hilbe, W., Weiss, W., Lukas, P., Jamnig, H., Hackl, M., Haidenberger, A., Buzewski, B., Miekisch, W., Schubert, J. and Amann, A. Noninvasive detection of lung cancer by analysis of exhaled breath. BMC Cancer 9:348. (2009)

7. Baltazar, M.F, Dickinson, F.M, and Ratledge, C. “Oxidation of medium-chain acyl-CoA esters by extracts of Aspergillus niger: emzymology and characterization of intermediates by HPLC,” Microbiology, vol. 145, no. 1, pp. 271–278 (1999).

8. Bard, D. "Traffic-related air pollution and the onset of myocardial infarction: disclosing benzene as a trigger? A small-area case-crossover study.". PLoS One. 9: 6. (2014)

9. Baselt, R.C. Biological Monitoring Methods for Industrial Chemicals. 2nd ed. Littleton, MA: PSG Publishing Co., Inc., p. 128 (1988)

10. Berg, J.M., Tymoczko, J.L. and Stryer, L. Biochemistry, international 7th ed. WH. Freeman company. Pages. 795-797. (2012)

11. Bloor, R.N., Španěl, P. and Smith, D. Quantification of breath carbon disulphide and acetone following a single dose of disulfiram (Antabuse) using selected ion flow tube mass spectrometry (SIFT-MS). Addiction Biology vol. 11 (2), p.163-169. (2006)

12. Broza, Y.Y, Zuri, L., and Haick, H. Combined Volatolomics for Monitoring of Human Body Chemistry. Scientific Reports 4: 4611 (2014)

13. Burg, J.S. and Espenshade, P.J. Regulation of HMG-CoA reductase in mammals and yeast. Progress in lipid research, 50, p. 403-410. (2011)

14. Buszewski, B., Ulanowska, A., Ligor, T., Jackowski, M., Klodzinska, E. and Szeliga, J. Identification of volatile organic compounds secreted from cancer tissues and bacterial cultures. Journal of Chromatography B, Volume 868, Issues 1–2, pp. 88-94 (2008).

15. Calenic, B., Filipiak, W., Greabu, M. and Anton Amann. Volatile Organic Compounds Expression in Different Cell Types: An In Vitro Approach. International Journal of Clinical Toxicology, 1, pp. 43-51. (2013)

16. Calenic, B., Miricescu, D., Greabu, M., Kuznetsov, A.V., Troppmair, J., Ruzsanyi, V and Amann, A. Oxidative stress and volatile organic compounds: interplay in pulmonary, cardio-vascular, digestive tract systems and cancer. Open Chem., 13: 1020–1030 (2015)

17. Carroll W, Lenney W, Wang T, Španel P, Alcock A, et al. Detection of volatile compounds emitted by Pseudomonas aeruginosa using selected ion flow tube mass spectrometry. Pediatric Pulmonology 39:452-456. (2005)

18. Chan AOO. Gastric cancer screening. In: UpToDate, Wong B (Ed), UpToDate. Accessed 29.01.17.
Available at:
https://datubazes.lanet.lv:2265/contents/gastriccancerscreening?source=search_result&search=gastric%20cancer%20screening&selectedTitle=1~18

19. Chen, X., Xu, F., Wang, Y., Pan, Y., Lu, D., and Wang, P. A study of the volatile organic compounds exhaled by lung cancer cells in vitro for breath diagnosis Cancer, 110 pp. 835-844 (2007)

20. Chu, Q.D, Gibbs, J.F and Zibari, G.B. Surgical Oncology: A Practical and Comprehensive Approach. Pages: 195-198. (2015)

21. Coppens, I. Targeting lipid biosynthesis and salvage in apicomplexan parasites for improved chemotherapies, Nature reviews. Microbiology, 11 (2013), pp. 823-835

22. DeBerardinis, R.J. and Chandel, N.S. Fundamentals of cancer metabolism. Sci. Adv. 2, e 1600200 (2016)

23. Downward, J. "Targerting RAS signaling pathways in cancer therapy". Nat. Rev. Cancer 3 (1) 11-22. (2003).

24. Ehmann R, Boedeker E, Friedrich U, Sagert J, Dippon J, Friedel G, Walles T. Canine scent detection in the diagnosis of lung cancer: revisiting a puzzling phenomenon. *Eur Respir J.* 39, 669-76 (2012).

25. Emi, Y., Sumiyoshi, Y., Oki, E., Kakeji, Y., Fukui, Y. and Maehara, Y. Pharmacokinetics of gamma-hydroxybutylic acid (GHB) and gamma-butyrolactone (GBL), the anti-angiogenic metabolites of oral fluoropyrimidine UFT, in patients with gastric cancer. Fukuoka Igaku Zasshi, 98 (12): 418-424. (2007)

26. Evaluation of certain food additives and contaminants (Forty-ninth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 884. (1999)

27. Fadda, S. and Talon, S.L. Preliminary characterization of β-decarboxylase activities in *Staphylococcus carnosus* 833, a strain used in sausage fermentation. *FEMS Microbiology Letters*, Volume 228, Issue 1, Pages 143–149 (2003)

28. Fadda, S., Lebert, A., Leroy-Sétrin, S., and Talon, R. "Decarboxylase activity involved in methyl ketone production by Staphylococcus carnosus 833, a strain used in sausage fermentation," FEMS Microbiology Letters, vol. 210, no. 2, pp. 209–214, (2002)

29. Fernandez, C., Lobo Md Mdel, V., Gomez-Coronado, D., and Lasuncion, M.A. Cholesterol is essential for mitosis progression and its deficiency induces polyploid cell formation. Exp. Cell Res., 300 (1), pp. 109-120 (2004).

30. Fernández del Río, R., O'Hara, M.E, Holt, A., Pemberton, P., Shah, T.,Whitehouse, T., and Mayhew, C.A. Volatile Biomarkers in Breath Associated With Liver Cirrhosis — Comparisons of Pre- and Post-liver Transplant Breath Samples. Ebiomedicine Vol. 2, Issue 9. Pages 1243-1250. (2015)

31. Filipiak, W., Sponring, A., Baur, M.M., Ager, C., Filipiak, A., Wiesenhofer, H., Nagl, M., Troppmair, J. and Amann, A. Characterization of volatile metabolites taken up by or released from Streptococcus pneumoniae and Haemophilus influenzae by using GC-MS. Microbiology, 158, 3044–3053 (2012)

32. Filipiak, W., Sponring, A. and Filipiak, A. "TD-GC-MS analysis of volatile metabolites of human lung cancer and normal cells in vitro," Cancer Epidemiology Biomarkers and Prevention, vol. 19, no. 1, pp. 182–195, 2010.

33. Fu, X.A., Li, M., Knipp, R.J., Nantz, M.H and.Bousamra, M. Noninvasive detection of lung cancer using exhaled breath. Cancer Med., 3, pp. 174-181 (2014)

34. Gallegos, J., Arce, C., Jordano, R., Arce, L. and Medina L.M. Target identification of volatile metabolites to allow the differentiation of lactic aci d bacteria by gas chromatography-ion mobility spectrometry. Food chemistry Vol. 220 Pages 362-370. (2017)

35. Garner CE, Smith S, Elviss NC, Humphrey TJ, White P, et al. (2008) Identification of Campylobacter infection in chickens from volatile faecal emissions. Biomarkers 13: 413-421.

36. Hakim, M, Broza, Y.Y., Barash, O., Peled, N., Phillips, M. and Amann, A.Volatile organic compounds of lung cancer and possible biochemicalpathways, Chem. Rev. 112 (11) 5949–5966. (2012)

37. Haick H, Broza YY, Mochalski P, Ruzsanyi V, Amann A. Assessment, origin, and implementation of breath volatile cancer markers. Chemical Society reviews 43, 1423–1449. (2014)

38. Hanai, Y., Shimono, K., Oka, H., Baba1, Y., Yamazaki, K. and Beauchamp, G.k. Analysis of volatile organic compounds released from human lung cancer cells and from the urine of tumor-bearing mice. Cancer Cell International 12:7. (2012)

39. Handa, H., Usuba, A., Maddula, S., Baumbach, J.I., Mineshita, M. and Miyazawa, T. Exhaled breath analysis for lung cancer detection using ion mobility spectrometry PLoS One, 9 (2014),

40. Harvey, R.A. and Ferrier D.R. Lippincott's Illustrated Reviews: Biochemistry Fifth Edition. pp. 190-197. (2011)

41. Hensley, C.T., Wasti, A.T. and DeBerardinis, R.J. Glutamine and cancer: Cell biology, physiology, and clinical opportunities. J. Clin. Invest. 123, 3678–3684 (2013).

42. Human Metabolome Database (HMDB)
Record Name: Gamma-Butyrolactone
URL: http://www.hmdb.ca/metabolites/HMDB0000549
Accessed 14.10.17

43. Human Metabolome Database (HMDB)
Record Name: N-Methylformamide
URL: http://www.hmdb.ca/metabolites/HMDB0001122
Accessed: 09.10.17

44. IARC. Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans. Geneva: World Health Organization, International Agency for Research on Cancer, 1972-PRESENT. (Multivolume work). Accessed on: 01.09.17 Available at: http://monographs.iarc.fr/ENG/Classification/index.php , p. V19 84 (1979)

45. IARC. Monographs on the evaluation of the Carcinogenic Risk of Chemicals to Humans. Available at: hhtp://monographs.iarc.fr/ENG/Classification/index.php, pV60 221 (1994)

46. IARC. Monographs on the "Re-evaluation of Some Organic Chemicals, Hydrazine and Hydrogen Peroxide". Proceedings of the IARC Working Group on the Evaluation of Carcinogenic Risks to Humans. Lyon, France.Volume 71 (1999)

47. Israelsen, W.J, Dayton, T.L, Davidson, S.M, Fiske, B.P, Hosios, A.M, Bellinger, G, Li, J, Yu, Y, Sasaki, M, Horner, J.W, Burga, L.N, Xie, J, Jurczak, M.J, DePinho, R.A, Clish, C.B, Jacks, T, Kibbey, R.G, Wulf, G.M, Di Vizio, D., Mills, G.B., Cantley, L.C. and Vander Heiden, M.G. PKM2 isoform-specific deletion reveals a differential requirement for pyruvate kinase in tumor cells. Cell **155**, 397–409 (2013)

48. Jackson, J.H., Cochrane, C.G., Bourne, J.R., Solski, P.A., Buss, J.E. and Der, C.J. Farnesol modification of Kristen-Ras Exon 4b-protein is essential for transformation. Proc. Natl. Acad. Sci. USA, 87 (8), pp. 3042-3046. (1990)

49. Jokelainen, K., Roine, R.P., Vaananen, H., Farkkila, M. and Salaspuro, M. In vitro acetaldehyde formation by human colonic bacteria. Gut; 35: 1271-1274 (1994)

50. Kapishon, V., Koyanagi, G.K., Blagojevic, V. and Bohme, D.K. Atmospheric pressure chemical ionization mass spectrometry of pyridine and isoprene: potential breath exposure and disease biomarkers. Journal of Breath Research 7 026005 (2013)

51. Kapoor, P., Deshmukh, R. and Kureja, I. GHB acid: A rage or reprieve. Journal of Advanced Pharmaceutical Technology and Research. Vol. 4 (4):173-178. (2013)

52. Karlic, H., Thaler, R., Germer, C., Grunt, T., Proestling, K., Haider, F. and Varga, F. Inhibition of the mevalonate pathway affects epigenetic regulation in cancer cells. Cancer genetics, vol. 208, issue 5, pp. 241-252. (2015)

53. Karpeh M, Kelsen D, Tepper J. Cancer of the stomach. In: DeVita JV, Hellman S, Rosenberg S, editors. Cancer: principles & practice of oncology, vol. 1. 6th ed. Philadelphia: Lippincott-Williams-Wilkin. p. 1092–126. (2001)

54. Kasper, Dennis L.et al. Harrison's Principles of Internal Medicine, 18th ed., McGraw-Hill Professional, p. 2017. (2012)

55. Keyomarsi, K., Sandoval, L., Band, V. and Pardee. A.B. Synchronization of tumor and normal cells from G1 to multiple cell cycles by lovastatin. Cancer Res., 51 pp. 3602-3609 (1991).

56. Kinsella, J.E and Hwang D.H, "Enzymes of Penicillium roqueforti involved in the biosynthesis of cheese flavor," CRC Critical Reviews of Food Science & Nutrition, vol. 8, no. 2, pp. 191–228, (1976)

57. Kischkel S, Miekisch W, Sawacki A, Straker EM, Trefz P, Amann A, Schubert JK. Breath biomarkers for lung cancer detection and assessment of smoking related effects – confounding variables, influence of normalization and statistical algorithms. Clin Chim Acta 411(21–22): 1637–1644. (2010)

58. Kostelc, J.G, Zelson, P.R., Preti, G. and Tonzetich, J. “Quantitative differences in volatiles from healthy mouths and mouths with periodontitis,” Clin Chem 27, 842-5 (1981).

59. Koppenol, W.H, Bounds, P.L and Dang, C.V. Otto Warburg’s contributions to current concepts of cancer metabolism. Nat. Rev. Cancer 11, 325–337 (2011).

60. Krilaviciute A., Heiss J. A., Leja M., Kupcinskas J., Haick H. and Brenner H.. Detection of cancer through exhaled breath: a systematic review. Oncotarget advance publications. (2015)

61. Kumar S. Thesis on: Mass Spectrometric Analysis of Volatile Organic Compounds in Oesophago-Gastric Cancer. Department of Surgery and Cancer, Imperial College London, St Mary’s Hospital, London, UK. (2014)

62. Kuzma, J., Nemecek-Marshall, M., Pollock, W.H. and Fall, R. Bacteria Produce the Volatile Hydrocarbon Isoprene. CURRENT MICROBIOLOGY Vol. 30, pp. 97-103 (1995)

63. Leja, M., Camargo, M. C., Polaka, I., Isajevs, S., Liepniece-Karele, I., Janciauskas, D., Rudzite, D., Kikuste, I., Vanags, A., Kojalo, I., Folkmanis, V., Kirsners, A., Tolmanis, I. & Rabkin, C. S. 2017. Detection of gastric atrophy by circulating pepsinogens: A comparison of three assays. *Helicobacter*.

64. Lewis, H.L and Darnall, D.W. “Fatty acid toxicity and methyl ketone production in Aspergillus niger,” Journal of Bacteriology, vol. 101, no. 1, pp. 65–71, (1970)

65. Ligor, M., Ligor, T., Bajtarevic, A., Ager, C., Pienz, M., Klieber, M., et al. Determination of volatile organic compounds in exhaled breath of patients with lung cancer using solid phase microextraction and gas chromatography mass spectrometry. Clin. Chem. Lab. Med., 47, pp. 550-560. (2009)

66. Ligor, T., Szeliga, J., Jackowski, M. and Buszewski, B.. Preliminary study of volatile organic compounds from breath and stomach tissue by means of solid phase microextraction and gas chromatography–mass spectrometry

J Breath Res, 1 (1) pp.1-6. (2007)

67. Likus, W., Siemianowicz, K., Bienk, K., Pakula, M., Pathak, H., Dutta, C., Wang, Q., Shojaei, S., Assaraf, Y.G., Ghavami, S., Cieslar-Pobuda, A and Los, M.J. Could drugs inhibiting the mevalonate pathway also target cancer stem cells? Drug resistance updates, vol. 25, pp. 13-25. (2016).
68.
69. Lindinger, W, Taucher J, Jordan A, Hansel A, Vogel W. Endogenous production of methanol after the consumption of fruit. Alcohol Clin Exp Res. 21(5):939-43 (1997)

70. Lubes G., and Goodarzi M. GC–MS based metabolomics used for the identification of cancer volatile organic compounds as biomarkers. Journal of Pharmaceutical and Biomedical Analysis. (2017)

71. Machado, R.F.; Laskowski, D.; Deffenderfer, O.; Burch, T.; Zheng, S.; Mazzone, P.J.; Mekhail, T.; Jennings, C.; Stoller, J.K.; Pyle, J., *et al.* Detection of lung cancer by sensor array analyses of exhaled breath. *Am. J. Respir. Crit. Care. Med.*, *171*, 1286–1291. (2005)

72. Mansfield PF. Clinical features, diagnosis, and staging of gastric cancer. In: UpToDate, Tanabe KK (Ed), UpToDate. Accessed 28.01.17. Available at: https://datubazes.lanet.lv:2265/contents/clinical-features-diagnosis-and-staging-of-gastriccancer?source=search_result&search=gastric%20cancer%20diagnostics&selectedTitle=1~150

73. Mathews, J.H, Raymer, A.S. Etheridge, G.R. Velez, J.R. Bucher. Do endogenous volatile organic chemicals measured in breath reflect and maintain CYP2E1 levels in vivo? Toxicol Appl Pharmacol.: 255-60 (1997)

74. Maurer-Stroh, S., Eisenhaber, F. Refinement and prediction of protein prenylation motifs. Genome Biol., 6 (6), p. R55 (2005)

75. Mayers, J.R., Wu, C., Clish, C.B., Kraft, P., Torrence, M.E., Fiske, B.P., Yuan, C., Bao, Y.,Townsend, M.K., Tworoger, S.S., Davidson, S.M., Papagiannakopoulos, T.,

Yang, A., Dayton, T.L., Ogino, S., Stampfer, M.J., Giovannucci, E.L., Qian, Z.R., Rubinson, D.R., Ma, J., Sesso, H.D., Gaziano, J.M., Cochrane, B.B., Liu, S., | Wactawski-Wende, J., Manson, J.E., Pollak, M.N., Kimmelman, A.C., Souza, A., Pierce, K., Wang, T.J., Gerszten, R.E., Fuchs, C.S.,Vander Heiden, M.G. and Wolpin, B.M. Elevation of circulating branched-chain amino acids is an early event in human pancreatic adenocarcinoma development. Nat. Med. 20, 1193–1198 (2014).

76. Mochalski P, Sponring A, King J., Unterkofler K., Troppmair J. and Amann A. Release and uptake of volatile organic compoundsby human hepatocellular carcinoma cells(HepG2) in vitro. Cancer Cell International, 13:72 (2013)

77. Moutinho, M., Nunes, M.J. and Rodrigues, E. The mevalonate pathway in neurons; It's not just about cholesterol. Experimental cell research. (2017)

78. Nagai, N., Mukai, K., Hirata, E., Jin, H.H, Komatsu, M. and Yunokawa, M. UFT and its metabolite gamma-butyrolactone (GBL) inhibit angiogenesis induced by vascular endothelial growth factor in advanced cervical carcinoma. Med. Oncol. 25 (2): 214-221. (2008)

79. Navaneethan, U., Parsi, M.A, Lourdusamy, V., Bhatt, A., Gutierrez, N.G., Grove, D., Sanaka, M.R., Hammel, J.P., Stevens, T., Vargo, J.J. and Dweik, R.A. Volatile organic compounds in bile for early diagnosis of cholangiocarcinoma in patients with primary sclerosing cholangitis: a pilot study. American Society for Gastrointestinal Endoscopy. Volume 81, No. 4 (2014)

80. Phillips, M., Cataneo, R.N, Cummin, A.R.C, Gagliardi, A.J., Gleeson, K., Greenberg, J. et al. Detection of lung cancer with volatile markers in the breath. Chest, 123 pp. 2115-2123. (2003)

81. Phillips, M.; Cataneo, R.N.; Saunders, C.; Hope, P.; Schmitt, P.; Wai, J. Volatile biomarkers in the breath of women with breast cancer. J. Breath Res. (2010)

82. Picton R, Eggo MC, Langman MJ, Singh S. Impaired detoxication of hydrogen sulfide in ulcerative colitis? Dig Dis Sci. 52, 373-378 (2007).

83. Plapp B.V, Leidal K.G, Murch B.P and Green D.W. Contribution of liver alcohol dehydrogenase to metabolism of alcohols in rats. Chemicobiological interactions vol. 235 p. 85-95. (2015)

84. Poli, D., Goldoni, M., Caglieri A. et al. "Exhaled volatile organic compounds in patients with non-small cell lung cancer: cross sectional and nested short-termfollow-up study," Respiratory Research, vol. 6, article 71, (2005)

85. Poli, D., Goldoni, M., Caglieri A. et al., "Breath analysis in non-small cell lung cancer patients after surgical tumour resection," Acta Biomedica de l'Ateneo Parmense, vol. 79, no.1, pp. 64–72, (2008).

86. Quigley, E.M.M. Gut bacteria in Health and Disease. Gastroenterology and Hepatology vol. 9, Issue 9. (2013)

87. Quintao, E.; Grundy, S.M.; Ahrens, E.H., Jr. Effects of dietary cholesterol on the regulation of total body cholesterol in man. *J. Lipid Res. 12*, 233–247. (1971)

88. Ramasamy S, Singh S, Taniere P, Langman MJ, Eggo MC. Sulfide-detoxifying enzymes in the human colon are decreased in cancer and upregulated in differentiation. Am J Physiol Gastrointest Liver Physiol. 291, 23 (2006)

89. Rochford, K., Chen, F, Waguespack, Y., Figliozzi, R.W., Kharel, M.K., Zhang, Q., Marin-Carabello, M. and Hsla, S.V. Volatile organic compound Gamma-Butyrolactone released upon Herpes Simplex Virus Type-1 Acute Infection Modulated Membrane Potential And Repressed Viral infection in Human Neuron-Like Cells. PLOS One. (2016)

90. Rudnicka, J., Kowalkowski, T., Ligor, T. and Buszewski, B. Determination of volatile organic compounds as biomarkers of lung cancer by SPME-GC-TOF/MS and chemometrics. Journal of Chromatography B, 879, p. 3360-3366. (2011)

91. Rudnicka, J., Walczak, M., Kowalkowski, T., Jezierski, T. and Buzewski, B. Determination of volatile organic compounds as potential markers of lung cancer by gas chromatography-mass spectrometry versus trained dogs. Sensors and Actuators B 202 615-621. (2014)

92. Sagar, N.M., Cree, I.A., Covington, J.A. and Aeasaradnam, R.P. The interplay of the Gut Microbiome, Bile Acids and Volatile Organic Compounds. Gastroenterology Research and Practice. Vol. 2015, ID 398585. (2015)

93. Sakumura, Y., Koyama, Y., Tokutake, H., Hida, T., Sato, K., Itoh, T., Akamatsu, T. and Shin, W. Diagnosis by Volatile Organic Compounds in Exhaled Breath from Lung Cancer Patients Using Support Vector Machine Algorithm. Sensors, vol. 17, Issue 2. (2017)

94. Schallschmidt, K.; Becker, R.; Zwaka, H.; Menzel, R.; Johnen, D.; Fischer-Tenhagen, C.; Rolff, J.; Nehls, I. in vitro cultured lung cancer cells are not suitable for animal-based breath biomarker detection. J. Breath Res., 9 (2) (2015).

95. Schallschmidt, K.; Becker, R.; Jung, C.; Rolff, J.; Fichtner, I., Nehls, I. Investigation of cell culture volatilomes using solid phase micro extraction: Options and pitfalls exemplified with adenocarcinoma cell lines. J. Chromatogr. B Analyt. Technol. Biomed. Life Sci., (2015)

96. Selyanchyn, R., Nozoe, T., Matsui, H., Kadosawa, T. and Lee, S.W. TD-GC-MS Investigation of the VOCs Released from Blood Plasma of Dogs with Cancer. Diagnostics, 3, 68-83. (2013)

97. Sherwood, L., Sherwood, W., Sherwood, J. and Woolverton, C. Prescotts Microbiology 9th ed. New York: McGraw Hill. pp. 713-721. (2013)

98. Shestivska V, Španel P, Dryahina K, Sovová K, Smith D, et al. Variability in the concentrations of volatile metabolites emitted by genotypically different strains of Pseudomonas aeruginosa. Journal of Applied Microbiology 113:701-713. (2012)

99. Singh, P., Saxena, R., Srinivas, G., Pande, G., Chattopadhyay, A., 2013. Cholesterol biosynthesis and homeostasis in regulation of the cell cycle. PLoS ONE, 8 (3) (2013)

100. Smith, M.T. "Advances in understanding benzene health effects and susceptibility". Annu Rev Publ Health. 31: 133–48. (2010)

101. Snyder, R. and Hedli, C.C. "An overview of benzene metabolism". Environ Health Perspect. **1**04 (6) pages. 1165–1171. (1996)

102. Sonoda H, Kohnoe S, Yamazato T, Satoh Y, Morizono G, Shikata K, Morita M, Watanabe A, Morita M, Kakeji Y, Inoue F, Maehara Y. Colorectal cancer screening with odour material by canine scent detection. *Gut.* 60, 814-9 (2011).

103. Sponring, A., Filipak, W., Mikoviny, T., Ager, C., Schubert, J., Miekisch, W., Amann, A. and Troppmair, J. Release of volatile organic compounds from the lung cancer cell lineNCI-H2087 in vitro. Anticancer Res. 29 (1), 419-426. (2009)

104. Statheropoulos, M., Agapiou, A. and Georgiadou, A. Analysis of expired air of fasting male monks at Mount Athos. Journal of Chromatography Analyt Technol Biomed Life Sci; 832:27 4–9. (2006)

105. Stone, B.G.; Besse, T.J.; Duane, W.C.; Evans, C.D.; DeMaster, E.G. Effect of regulating cholesterol biosynthesis on breath isoprene excretion in men. *Lipids, 28*, 705–708. (1993)

106. Suarez F, Furne J, Springfield J, Levitt M. Insights into human colonic physiology obtained from the study of flatus composition. Am J Physiol. 272,1028-33. (1997)

107.Sulë-Suso, J., Pysanenko, A., Spanel, P. and Smith, D. Quantifiaction of acetaldehyde and carbon dioxide in the headspace of malignant and non-malignant lung cells in vitro by SIFT-MS. Analyst (Lond.) 134 (12). (2009)

108.Szaboa C., Colettaa C., Chaob C., Módisa K., Szczesnyc B., Papapetropoulosa A., and Hellmichb M.R. Tumor-derived hydrogen sulfide, produced by cystathionine-β-synthase, stimulates bioenergetics, cell proliferation, and angiogenesis in colon cancer. PNAS vol. 110 no. 30. (2013)

109.Tait, E., Perry, J.D., Stanforth, S.P and Dean J.R. Identification of Volatile Organic Compounds Produced by Bacteria Using HS-SPME-GC-MS. Journal of Chromatograpic Science, vol 52 pp. 363-373. (201

110. Tangerman, A. Measurement and biological significance of the volatile sulfur compounds hydrogen sulfide, methanethiol and dimethyl sulfide in various biological matrices. J Chromatogr B Analyt Technol Biomed Life Sci; 877 (28):3366-77. (2009)

111.Tavares, C.D.J, Sharabi, K., Dominy J.E., Lee., Y., Isasa, M., Orozco, J.M., Jedrychowski, M.P., Kamenecka, T.M., Griffin, P., Gygi, S.P. and Puigserver, P. The Methionine Transamination Pathway Controls Hepatic Glucose Metabolism through Regulation of the GCN5 Acetyltransferase and the PGC-1_ Transcriptional Coactivator. The Journal of Biological Chemistry Vol. 291, NO 20, pp. 10635-10645. (2016)

112.Trefz P, Koehler H, Klepik K, Moebius P, Reinhold P, et al. Volatile emissions from Mycobacterium avium subsp. paratuberculosis mirror bacterial growth and enable distinction of different strains. PLoS One 8: e76868. (2013)

113.Ulanowska, A., Ligor, T., Amann, A. and Buszewski, B. Evaluation of Septa Quality for Automatic SPME–GC–MS: Trace Analysis. Journal of Chromatographic Science; 50:10–14 (2012)

114. Väkeväinen S, et al. Acetaldehyde production and other ADH-related characteristics of aerobic bacteria isolated from hypochlorhydric human stomach. Alcohol Clin Exp Res; 25:421-426. (2001)

115. Van den Velde S., Quirynen M., Hee P. V. and Steenberghe D.V. Halitosis associated volatiles in breath of healthy subjects. Journal of Chromatography B, 853 54–61 (2007)

116. Van den Velde, S, Nevens, F., Van hee P., van Steenberghe D., and Quirynen M. GC–MS analysis of breath odor compounds in liver patients. Journal of Chromatography, vol 875, issue 2, Pages 344-348. (2008)

117. Walker, V., Begley, JP. and Stansbridge, E. M. "2-Pentanone: an indicator of peroxisomal acyl-CoA thioesterase activity?" Journal of Inherited Metabolic Disease, vol. 31, supplement 1, p. 67. (2008)

118. Walker, V. and Mills G. 2-Pentanone Production from Hexanoic Acid by Penicillium roqueforti from Blue Cheese: Is This the Pathway Used in Humans? The Scientific World Journal Volume 2014. Article ID 215783 (2014)

119. Walsh, C.T., Garneau-Tsodikova, S. and Howard-Jones., A.R. Biological formation of pyrroles: Nature's logic and enzymatic machinery. The Royal Society of Chemistry *Nat. Prod. Rep.*, *23*, 517–531 (2006)

120. World Health Organization. Cancer: Fact Sheet No 297. WHO. Available at http://www.who.int/mediacentre/factsheets/fs297/en/. Accessed: 28.01.2017

121. World Health Organization. Cancer: Fact Sheet, Stomach cancer. WHO. Available at: http://globocan.iarc.fr/Pages/fact_sheets_cancer.aspx Accessed: 31.01.2017

122. Wu, C.F., Uang, S.N., Chiang, S.Y., Shih, W.C., Huang, Y.F and Wu, K.Y. Simultaneous quantitation of urinary cotinine and acrylonitrile-derived mercapturic acids with ultraperformance liquid chromatography–tandem mass spectrometry. Analytical and Bioanalytical Chemistry. Vol 402. Issue 6. Pages 2113-2120. (2012)

123. Xu, Z., Broza, Y.Y, Lonsecu R, Tisch U, Ding U, Liu H, Song Q, Pan Y-y, Xiong F-x, Gu K-s, Sun G-p, Chen Z,d, Leja M. and Haick H, (2013). A nanomaterial-based

breath test for distinguishing gastric cancer from benign gastric conditions. British Journal of Cancer 108, 941–950 (2013)

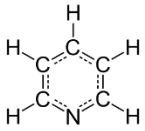
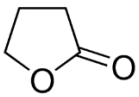
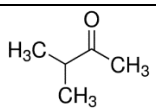
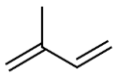
124. Yamagishi K., Onuma K., Chiba Y., Yagi S., Aoki S., Sato T., Sugawara Y., Hosoya N., Saeki Y., Takahashi M., Fuji M., Ohsaka T., Okajima T., Akita K., Suzuki T., Senawongse P., Urushiyama A., Kawai K., Shoun H., Ishii Y., Ishikawa H., Sugiyama S., Nakajima M., Tsuboi M. and Yamanaka T. Generation of gaseous sulfur-containing compounds in tumour tissue and suppression of gas diffusion as an antitumour treatment. British Society of Gastroenterology 554-61 (2012).

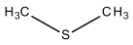
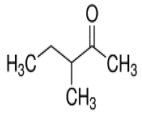
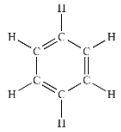
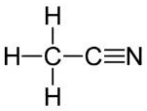
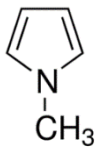
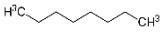
125. Yeganeh, B., Wiechec, E., Ande, S.R., Sharma, P., Moghadam, A.R. and Post M. Targeting the mevalonate cascade as a new therapeutic approach in heart disease, cancer and pulmonary disease. Pharmacol. Ther., 143 (1), pp. 87-110 (2014).

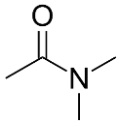
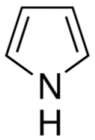
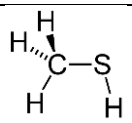
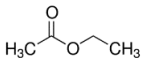
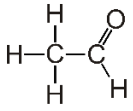
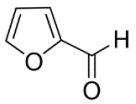
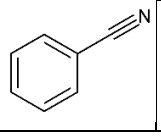
126. Zhu J, Bean HD, Kuo YM, Hill JE. Fast detection of volatile organic compounds from bacterial cultures by secondary electrospray ionization-mass spectrometry. J ClinMicrobiol 48: 4426-4431. (2010)

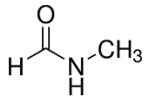
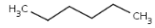
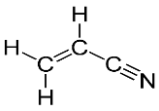
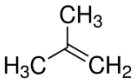
Appendix

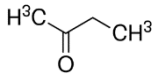
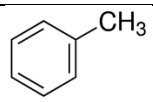
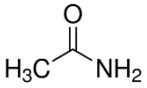
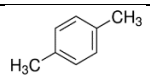
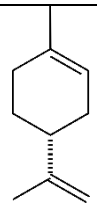
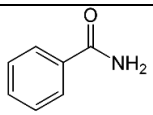
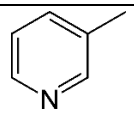
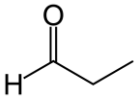
Table 4 Shows list of emitted compounds, their potential origin and relation to VOCs found in literature. The compounds in blue were found significantly different in this study.

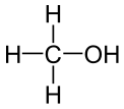
Compound	Formula	Group / class	Increase/ decrease in cancer	Potential origin				Notes:	References:
				Cell metabolism	Microbiome	Environment/diet / drugs	Oxidative stress		
Carbon disulfide	S=C=S	VSC	↑	Yes (Methionine, cysteine, cystine)	Yes (S. pneumonia, H. influenza, S. aureus, P. aeruginosa)	Yes (Environmental air, disulfiram therapy)	Yes (Methionine, Cysteine, cystine)	Found in lung cancer and gastric cancer	*Buzewski et al. (2012), Buzewski et al. (2008) Rudnicka et al. (2011), Ligor et al. (2007), Blor (2006)*
Pyridine		Aromatic nitrogen compound	↑	-	-	Yes (Cigarette smoke, diet)	-	Found in lung cancer. Also found in people with periodontal disease.	*Filpak et al. (2014), Kostelc et al. (1981)*
Butyrolactone		Lactone	-	Yes (Can be produced endogenously from GABA)	-	Yes (Fluoropyrimidines could be metabolized into GBL)	-	-	*Human metabolome database: Gamma-Butyrolactone, Nagai et al. (2008), Emi et al. (2007)*
2-Butanone, 3-methyl		Ketone	-	Yes (Lipid metabolism)	Yes (P. aeruginosa)	-	-	-	*(Shestivska et al. (2012)*
Isoprene		Hydrocarbon	↓↑	Yes (Mevalonate pathway)	Yes (M. avium, bacillus)	-	-	↑↓ Lung cancer, ↓ hepatocellular carcinoma, ↑ Gastric cancer and	*Rudnicka et al. (2011), Rudnicka et al. (2014) Bajartevic et al (2009), Mochalski et al. (2013), Xu et al. (2013), Chen (2007),*

								peptic ulcer disease	*Ulanowska et al. (2011), Trefz et al. (2013), Kuzma et al. (1995)*
Dimethyl sulfide		VSC	↑	Yes (Cyteine and Methionine metabolism)	Yes (P. aeruginosa, M. avium, H. pylori, M. avium)	-	Yes (ROS methionine reaction)	Hepatocellular carcinoma, Lung cancer,	*Mochalski et al. (2013), Rudnicka et al. (2011), Rudnicka et al. (2014), Buzewski et al. (2008), Ulanowska et al. (2011), Shestivska et al. (2012), Trefz et al. (2013)*
2-Pentanone		Ketone	↑	Yes (Lipid metabolism)	Yes (Pseudomonas, Pneumococci, H. Influenza, S. aureus)	Yes Diet (fruits)	-	Found in lung cancer, Hepatocellular carcinoma.	*Hanai et al. (2012), Buzewski et al. (2012), Filipak et al. (2010), Ulanowska et al. (2011), Shestivska et al. (2012)*
Benzene		Hydrocarbon	↓↑	-	Yes (H.pylori, M. avium)	Yes Cigarette smoke, pollution	-	Found in lung cancer. Was decreased after resection of lung cancer. Increased in smokers.	*Poli et al. (2005), Poli et al. (2008), Hakim et al. (2012), Chen et al. (2007), Ulanowska et al. (2011), Trefz et al. (2013)*
Acetonitrile		Nitrile	↑↓	-	Yes (E. coli, S. aureus)	Yes (smoking)	-	Lung cancer	*Filipak et al. (2008), Rudnicka et al. (2011), Zhu et al. (2010)*
Pyrrole, 1-methyl		Aromatic nitrogen compound	-	-	Yes (M. avium)	-	-	-	*Tretz et al. (2013)*
n-Octane		Alkane	↑↓	Yes (lipid peroxidation)	Yes (H. Pylori, M. avium)	-	-	↑↓ Lung cancer	*Filipak et al. (2014), Filipak et al. (2010), Schallscmidt et al. (2015), Poli et al. (2005), Ulanowska et al. (2011),*

									Buzewski et al. (2008), Trefz et al. (2013)
Acetamide, N,N-dimethyl-		Nitriles	↑	-	-	-	-	Found to be increased in small amounts in some Tedlar bags used to collect breath samples.	*(Calenic et al (2013), Bajtarevic et al (2009) and Altomare et al. (2013)*
Pyrrole		Aromatic nitrogen compound	↑	Yes Pyrrole is used as a building block for haemoglobin, vitamin B12, tryptophan	Yes C. Jejuni	-	-	↑ Lung cancer, ↑in plasma of dogs with different cancers.	*Filipak et al. (2010), Selyanchym et al (2013), Garner et al. (2008)*
Methanethiol (Mercaptomethane)		VSC	↑	Yes (Methionine, Cysteine metabolism)	Yes (S. Pneumoniae, H. Influenza, H. Pylori)	-	Yes (ROS reaction with Methionine and Cysteine)	-	*Filipak et al. (2012), Ahn et al. (2016), Ulanowska et al. 2011, Buzewski et al. (2008), Tangerman (2009)*
Ethyl Acetate		Ester	↑	-	-	Yes common solvent	-	Lung cancer	*Schallschmidt et al. (2015)*
Acetaldehyde		Aldehyde	↓↑	Yes (Lipid peroxidation, ethanol metabolism)	-	Yes (Can be the product of metabolism of exogenous ethanol) (disinfectant)	-	Lung cancer, Gastric and Oesophageal cancer	*Sulé-Suso et al. (2009), Sponring et al. (2009), Filipak et al. (2008), Jokelainen et al. Gut (1994), 35, 1271* Väkeväinen et al. (2001), *Kumar (2012)*
Furfural		Furan aldehyde	↑	-	-	-	-	Gastric cancer and peptic ulcer disease	*Xu et al. (2013)*
Benzonitrile		Nitrile	-	-	-	-	-	No relevant studies found.	-

Formamide, N-methyl-		Formamide	-	-	-	Yes (N, N-Dimethylformamide (DMF) exposure)	Yes (It has been shown to deplete glutathione, a key antioxidant)	Dimethylformamide is metabolized by sequential N-demethylation to methyl formamide and formamide that are excreted in the urine	*Human metabolome database, Baselt (1988)*
n-Hexane		Alkane	↑	Yes (Lipid peroxidation)	Yes (M. avium)	Yes, (solvent)	-	Lung cancer	*Ligor et al. (2009), Handa et al. (2014), Trefz et al. (2013)*
2-Propenenitrile (Acrylonitrile)		Nitriles	↓↑	-	-	Yes (Cigarette smoke, car exhaust. Considered environmental pollutant and potential carcinogen. Speculation on storage in tissue)	-	↑ Gastric cancer, ↓ Oseophageal adenocarcinoma, ↑ Lung cancer, ↑ PSG	*Xu et al. (2013), Bhatt et al. (2015), (Kischkel et al, 2010), Navaneethan et al. (2015)*
1-Propene, 2-methyl-		Hydrocarbon	-	-	Yes (H. Pylori)	-	-	-	Ulanowska et al. (2011), Buzewski et al. (2008)
n-Heptane		Alkane	↓	Yes (Thought to be due to lipid peroxidation)	Yes (M. avium)	-	-	Lung cancer	*Schallscmidt et al. (2015), Poli et al. (2005), Phillips et al. (2003), Trefz et al. (2013)*
n-Pentane		Alkane	↓	Yes (Thought to be due to lipid peroxidation)	Yes (H. Pylori, M. avium)	-	-	Lung cancer	*Schallschmidt et al. (2015), Phillips et al. (2003), Ligor et al. (2003), Ulanowska et al. (2011), Buzewski et al. (2008), Trefz et al. (2013)*

2-Butanone		Ketone	↓↑	Yes (Lipid metabolism)	Yes (H.Pylori, P. aeruginosa, M. avium)	Yes (solvent)	-	Found in lung cancer ↑ in adenocarcinoma ↓ non-small cell carcinoma, ↑ gastric cancer, ↑ Ovarian cancer, ↑ Liver cirrhosis, H. pylori positive patients	*Hanai et al. 2015, Buzewski et al. (2012), Zhang et al. (2014), Filipak et al. (2008), Amal et al. (2014), Van Den Velde et al. (2008), Fernandez Del Rio et al. (2015), Sohrabi et al. (2014), Ligor et al.(2009), Fu et al. (2014), Shestivska et al. (2012), Trefz et al. (2013).*
Toulene (Methylbenzene)		Aromatic hydrocarbon	↑	-	Yes (H. Pylori)	Yes (Cigarette smoke, pollution)	-	↑ Lung cancer	*Hakim et al. (2012), Poli et al. (2005), Ulanowska et al. (2011), Buzewski et al. (2008)*
Acetamide		Amide	-	-	-	Yes (solvent/ plasticizer.)	-	No relevant studies found	-
p-Xylene		Aromatic hydrocarbon	↓	-	-	Yes (Cigarette smoke, pollution)	-	↓ Lung cancer	*Schallschmidt et al. (2015)*
DL-Limonene		Cyclic terpene	↑	-	-	Yes (diet/fruits)	-	↑ Breast cancer	*Phillips et al. (2010)*
Benzamide		Aromatci hydrocarbon	-	-	-	-	-	No relevant studies found	-
Pyridine, 3-methyl-		Aromatic nitrogen compound	-	-	-	-	-	No relevant studies found	-
n-Propanal		Aldehyde	↓	Yes (Lipid peroxidation)	-	1-propanol metabolism (from disinfection?)	-	Lung cancer	*Schallschmidt et al. (2015)*

						-			
Methanol		Alcohol	↑	Metabolized to formaldehyde which has been found in gastric and esophageal adenocarcinomas.	Formed by anaerobic metabolism of several bacteria, including P. aeruginosa	Metabolism of artificial sweetener Aspartame, metabolism of pectin from fruit consumption	-	Lung cancer, Gastric cancer, Oesophageal cancer	*Machado et al. (2005), Bajtarevic et al. (2009), Carroll et al. (2005), Kumar et al. (2012), Kumar (2014), Lindinger et al. (1997)*

PSG- Primary Sclerosing Cholangitis

VSC-Volatile Sulfur Compound

GABA- Gamma-aminobutyrate

afonds.ētika
MEDICĪNISKO UN BIOMEDICĪNISKO PĒTĪJUMU ĒTIKAS KOMITEJA

Darbojas saskaņā ar SHK LKP noteikumiem

Nr. 28-A/15
05.11.2015.
Rīgā

Rīgas Austrumu klīniskās universitātes slimnīcas atbalsta fonda
Medicīnisko un biomedicīnisko pētījumu Ētikas komitejas

ATZINUMS

Pētījuma nosaukums : Gaistošo organisko marķieru noteikšana audu materiālā un bioloģiskajos šķidrumos, to korelācijas noteikšana ar izelpojamā gaisā rodamajiem marķieriem

Pētījuma pieteikuma iesniedzējs: Mārcis Leja

Pētījuma pieteikuma iesniedzēja darba vieta: RAKUS

SIA "Rīgas Austrumu klīniskās universitātes slimnīcas" atbalsta fonda Medicīnisko un biomedicīnisko pētījumu Ētikas komiteja(sēdes prot.12/15., 05.11.15.) ir izvērtējusi plānotā zinātniskā pētījuma nozīmi un mērķi, iesniedzēja sniegto paredzamā ieguvuma un riska novērtējumu un tā pamatotību. Balstoties uz iesniegto dokumentu izvērtējumu, komiteja nolēma izteikt:

- ■ pozitīvu atzinumu
- ☐ negatīvu atzinumu, ar iespēju veikt izmaiņas un iesniegt pieteikumu atkārtoti
- ☐ negatīvu atzinumu

par pieteikuma atbilstību zinātnisko pētījumu ētikas prasībām.

Rīgas Austrumu klīniskās universitātes slimnīcas atbalsta fonda
Medicīnisko un biomedicīnisko pētījumu
Ētikas komitejas priekšsēdētājs Roberts Stašinskis

DOCUMENTATION PAGE

This Diploma Thesis

„_____
_____
_____”

was developed at the Faculty of Medicine of the University of Latvia.

With my signature, I attest, that this research has been carried out without aid or assistance. Used information was obtained only from indicated sources and the electronically submitted copy of this diploma work complies with printout.

Author: _____ _____

(name, surname) (*signature)*

I recommend the work for presentation.

Supervisor: _____ _____ _____

(position, name, surname, degree) *(siganture)* *(date)*

Reviewer: _____ _____ _____

(position, name, surname, degree) *(signature)* *(date)*

The diploma thesis was submitted to the Faculty of Medicine on: _____

(date)

International students' coordinator, Inese Vēvere _____

(signature)

The diploma thesis is presented at the meeting of the State Examination Commission of Higher Professional Study Program „Medicine”_____2017. Protocol No. _____

Secretary of Commission: _____ _____

(position, name, surname, degree) *(signature)*