Volatile organic compounds in health and disease: a breathtaking breakthrough?

Kevin Lamote, PhD  
kevin.lamote@uantwerpen.be

Research Club  
October 4, 2017
Overview

• The world of "-omics"

• Volatile Organic Compounds (VOCs)
  • Origin
  • Breath biopsy
  • Stool, blood, tissue

• Technology

• Applications
  • Biomarker
  • Precision medicine
  • Patient selection
  • Monitoring
  • Early detection/screening

• Future perspectives
The world of "-omics"
Breath analysis
Volatile Organic Compounds (VOCs)

Van der Schee et al. Chest. 2015
Historical breath analysis

ANNUAL NUMBER OF BREATH VOC PUBLICATIONS

- volatile organic compound AND breath

YEAR

NUMBER OF PUBLICATIONS

VOC analysis
Key factors – What to consider

• Sampling
  • Online
  • Offline
    • Bags (Tedlar, Mylar)
    • Canisters (Stainless steel)
    • Adsorbent tubes (Tenax\textsuperscript{GR})
    • Solid phase microextraction (SPME) fibers

• Sources

<table>
<thead>
<tr>
<th></th>
<th>Breath</th>
<th>Blood</th>
<th>Urine</th>
<th>Stool</th>
<th>Skin</th>
<th>cell lines/bacteria</th>
</tr>
</thead>
</table>
Breath Analysis

Techniques

Van der Schee et al. Chest. 2015
Breath Analysis Techniques

- Gas chromatography – mass spectrometry (GC-MS)
Breath Analysis Techniques

• Gas chromatography – mass spectrometry (GC-MS)

Lamote K et al. Cancer Epidemiol Biomark Prev, 2014
Breath Analysis Techniques

- Electronic nose (eNose)
Breath Analysis Techniques

- Multicapillary column - ion mobility spectrometry (MCC/IMS)

Lamote K et al. Cancer Epidemiol Biomark Prev, 2014
Breath Analysis Techniques

- Animals
# Breath Analysis Methods

<table>
<thead>
<tr>
<th>GC-MS</th>
<th>eNose</th>
<th>IMS</th>
<th>Canine scent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitive (pp&lt;sub&gt;m&lt;/sub&gt;-pp&lt;sub&gt;v&lt;/sub&gt;)</td>
<td>No Specific VOC identification</td>
<td>Sensitive (pp&lt;sub&gt;b&lt;/sub&gt;-pp&lt;sub&gt;v&lt;/sub&gt;)</td>
<td>Time consuming (dog training)</td>
</tr>
<tr>
<td>Identification, detection, quantification of VOCs</td>
<td>Black box</td>
<td>VOC identification possible with MCC column</td>
<td>No quantification/identification of VOCs</td>
</tr>
<tr>
<td>Vacuum conditions</td>
<td>Ambient conditions</td>
<td>Ambient conditions</td>
<td>Ambient conditions</td>
</tr>
<tr>
<td>Slow</td>
<td>Fast, easy</td>
<td>Fast, easy</td>
<td>Fast, easy</td>
</tr>
<tr>
<td>Offline sampling</td>
<td>Offline sampling</td>
<td>Online sampling</td>
<td>Online sampling</td>
</tr>
<tr>
<td>Large, immovable set-up</td>
<td>Transportable</td>
<td>Transportable</td>
<td>Transportable</td>
</tr>
<tr>
<td>Very expensive</td>
<td>Cheap</td>
<td>Cheap</td>
<td>Expensive</td>
</tr>
<tr>
<td>Specific technician training</td>
<td>No specific operator training</td>
<td>No specific operator training</td>
<td>No specific operator training</td>
</tr>
<tr>
<td>Gold standard</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: pp<sub>b</sub>, parts per billion by volume; pp<sub>m</sub>, parts per million by volume; pp<sub>v</sub>, parts per trillion by volume.
Breath Analysis
Applications

• Clinical diagnostics and prognosis (*infections*)
• Early detection and screening (*cancer*)
• Disease monitoring (*COPD exacerbations*)
• Precision medicine
  • Companion diagnostics
  • Patient stratification (*eos-neutro asthma, cancer mutations ...*)
  • Treatment response (*corticosteroid response*)
  • Toxicity (*radiation, pollution*)
# Breath Analysis

## Approved applications

<table>
<thead>
<tr>
<th>Test</th>
<th>VOC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Capnography</td>
<td>CO₂</td>
</tr>
<tr>
<td>¹³C-urea breath test for <em>H. pylori</em> infection</td>
<td>¹³CO₂</td>
</tr>
<tr>
<td>Neonatal jaundice</td>
<td>CO</td>
</tr>
<tr>
<td>Disaccharaide adsorption deficiency</td>
<td>H₂, CH₄</td>
</tr>
<tr>
<td>Monitoring asthma therapy</td>
<td>FeNO</td>
</tr>
<tr>
<td>Heart transplant rejection</td>
<td>Alkanes</td>
</tr>
<tr>
<td>¹³C-octanoic acid Gastric Emptying Breath test</td>
<td>¹³CO₂</td>
</tr>
<tr>
<td>CO testing smokers</td>
<td>CO</td>
</tr>
<tr>
<td>Roadside intoxication</td>
<td>CH₃CH₂OH</td>
</tr>
</tbody>
</table>
Breath Analysis
Applications

Breath VOCs reported for a wide range of diseases:

- Head and neck cancer
- Asthma and COPD
- Pulmonary embolism
- Breast cancer
- Acute respiratory distress syndrome
- Artherosclerosis
- Liver cancer
- Liver cirrhosis
- Alcohol hepatitis
- Non-alcohol fatty liver disease
- Diabetes
- Inflammatory bowel disease
- Schizophrenia
- Tuberculosis
- Mesothelioma
- Lung cancer
- Cystic fibrosis
- Heart disease
- Gastric cancer
- H. pylori infection
- Renal failure
- Carbohydrate malabsorption
- Colorectal cancer
- Rheumatoid arthritis
Breath Analysis
Asthma

- 63 asthmatic children
- 57 healthy control children
- 95% specificity, 89% sensitivity, 92% accuracy

Table 2. Overview of the most important components used to discriminate asthma patients from healthy controls

<table>
<thead>
<tr>
<th>Number*</th>
<th>Component</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>(Branched) hydrocarbon (C_{13}H_{28})</td>
</tr>
<tr>
<td>2</td>
<td>Carbon disulphide (CS_{2})</td>
</tr>
<tr>
<td>3</td>
<td>1-penten-2-on</td>
</tr>
<tr>
<td>4</td>
<td>butanoic acid</td>
</tr>
<tr>
<td>5</td>
<td>3-(1-methylethyl)-benzene</td>
</tr>
<tr>
<td>6</td>
<td>(Branched) hydrocarbon (C_{13}H_{28})</td>
</tr>
<tr>
<td>7</td>
<td>Unsaturated hydrocarbon (C_{15}H_{26})</td>
</tr>
<tr>
<td>8</td>
<td>Benzoic acid</td>
</tr>
<tr>
<td>9</td>
<td>p-xylene</td>
</tr>
<tr>
<td>10</td>
<td>(Branched) hydrocarbon (C_{11}H_{24})</td>
</tr>
</tbody>
</table>

*Numbers represent the order in which the components were included in the discriminant analysis.

Dallinga et al. Clinical & Experimental Allergy, 2009
Breath Analysis
Asthma

• 10 young patients with mild asthma
• 10 older patients with severe asthma
• 10 young / 10 older healthy controls

• Mild vs healthy: 100%
• Severe vs Healthy: 90%
• Mild vs severe: 65%
Breath Analysis
Asthma

- Phenotyping:
  - 35 asthmatic patients
  - 23 matched healthy controls

Table 3: Receiver operating characteristics of the multivariate models for the asthma phenotypes of interest

<table>
<thead>
<tr>
<th></th>
<th>Eosinophilic versus non-eosinophilic</th>
<th>Neutrophilic versus non-neutrophilic</th>
<th>Controlled versus not controlled</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity</td>
<td>0.75</td>
<td>0.80</td>
<td>0.89</td>
</tr>
<tr>
<td>Specificity</td>
<td>0.90</td>
<td>0.75</td>
<td>0.88</td>
</tr>
<tr>
<td>Positive predictive value</td>
<td>0.86</td>
<td>0.80</td>
<td>0.89</td>
</tr>
<tr>
<td>Negative predictive value</td>
<td>0.82</td>
<td>0.75</td>
<td>0.88</td>
</tr>
<tr>
<td>AUROC (95% CI)</td>
<td>0.98 (0.91 to 1.00)</td>
<td>0.90 (0.76 to 1.00)</td>
<td>0.97 (0.93 to 1.00)</td>
</tr>
<tr>
<td>Cross-validation accuracy</td>
<td>83%</td>
<td>72%</td>
<td>80%</td>
</tr>
</tbody>
</table>

Leave-one-out cross-validation accuracy from discriminant function analysis also shown.
AUROC, area under the receiver operating characteristic.

Figure 2: Receiver operating characteristic curves for the models predicting eosinophilic versus non-eosinophilic asthma (solid line); neutrophilic versus non-neutrophilic asthma (dashed line) and controlled versus uncontrolled asthma (dotted line).

Ibrahim et al. Thorax, 2011
Breath Analysis
Asthma

• Steroid responsiveness:
  • asthmatic patients (11 steroid responsive, 7 unresponsive)
Breath Analysis COPD

- 30 COPD patients
- 20 asthmatic patients
- 40 controls (20 non-smoking and 20 smoking)

Fens et al. Am J Respir Crit Care Med, 2009
Breath Analysis
COPD

• Infections in acute exacerbations:
  • 43 COPD patients
    • 22 bacterial infections, 18 viral infections (9 both viral and bacterial infections)
    • 12 no infections

Breath Analysis
COPD

- COPD vs Lung Cancer:
  - 10 Lung cancer, 10 COPD

Acc: 85%

Dragonieri et al. Lung Cancer, 2009
Breath Analysis
Oncology – Lung cancer

• Poor prognosis
• Early detection proven to increase patients outcome
  • Low dose CT screening reduced mortality by 20%
• Breath analysis for screening for cancer?
<table>
<thead>
<tr>
<th>Group</th>
<th>Method</th>
<th>Patients</th>
<th>Controls</th>
<th>Model Characteristics</th>
<th>VOCs</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gordon et al. (1985)</td>
<td>TD-GC-MS</td>
<td>12 LC</td>
<td>17 HC</td>
<td>Acc: 93%</td>
<td>Acetone, methylethylketone, n-propanol</td>
<td></td>
</tr>
<tr>
<td>Phillips et al. (1999)</td>
<td>TD-GC-MS</td>
<td>60 LC</td>
<td>48 HC</td>
<td>Se: 71.7% Sp: 66.7%</td>
<td>Styrene, 2,2,4,6,6-pentamethyheptane, 2-methylheptane, Decane, propylbenzene, Undecane, methylcyclopentane, 1-methyl-2-pentylcyclopropane, trichlorofluoro-methane, benzene, 1,2,4-trimethyl-benzene, isoprene, 3-methyloctane, 1-hexene, 3-methylnonane, 1-heptene, 1,4-dimethylbenzene, 2,4-dimethyl-heptane, Hexanal, Cyclohexane, 1-methylcyclopentyl-benzene, Hepatanal</td>
<td></td>
</tr>
<tr>
<td>Phillips et al. (1999)</td>
<td>TD-GC-MS</td>
<td>82 LC</td>
<td>91 non-cancer 41 HC</td>
<td>Se: 85.1% Sp: 80.5%</td>
<td>C₄-C₂₀ monomethylated alkanes</td>
<td>No effect stage, smoking, histology</td>
</tr>
<tr>
<td>Phillips et al. (2003)</td>
<td>TD-GC-MS</td>
<td>82 LC</td>
<td>91 non-cancer 41 HC</td>
<td>Se: 85.1% Sp: 80.5%</td>
<td>2-methylpentane, isoprene, pentane, ethylbenzene, xylene, trimethylbenzene, toluene, benzene, heptane, decane, styrene, octane, pentamethylheptane</td>
<td></td>
</tr>
<tr>
<td>Poli et al. (2005)</td>
<td>SPME-GC-MS</td>
<td>36 NSCL 25 COPD</td>
<td>35 smokers 50 non-smokers</td>
<td>Se: 80%</td>
<td>Acetonitrile, 2,5-dimethylfuran, benzene</td>
<td></td>
</tr>
<tr>
<td>Ligor et al. (2009)</td>
<td>SPME-GC-MS</td>
<td>65 LC</td>
<td>31 Controls</td>
<td>-</td>
<td>2-methylpentane, 1-propanol, 3-butyn-2-ol, benzaldehyde, 3-methylpentane, n-pentane, n-hexane, 2-butanone</td>
<td>Acetonitrile, 2,5-dimethylfuran, benzene ~smoking</td>
</tr>
<tr>
<td>Chen et al. (2007)</td>
<td>SPME-GC-MS</td>
<td>29 LC</td>
<td>13 HC</td>
<td>Se: 86.2% Sp: 69.2%</td>
<td>Styrene, decane, isoprene, benzene, Undecane, 1-hexene, Hexanal, Propylbenzene, 1,2,4-trimethylbenzene, Heptanal, Methylcyclopentane</td>
<td></td>
</tr>
<tr>
<td>Phillips et al. (2007)</td>
<td>SPME-GC-MS</td>
<td>193 LC</td>
<td>211 Controls</td>
<td>Se: 84.6% Sp: 80%</td>
<td>C₁₄-C₂₄ hydrocarbons</td>
<td>Independent of smoking, stage</td>
</tr>
<tr>
<td>Phillips et al. (2008)</td>
<td>SPME-GC-MS</td>
<td>193 LC</td>
<td>211 Controls</td>
<td>Se: 84.5% Sp: 81%</td>
<td>C₁₄-C₂₄ hydrocarbons</td>
<td>WDA, No influence of smoking</td>
</tr>
<tr>
<td>Gaspar et al. (2009)</td>
<td>SPME-GC-MS</td>
<td>30 LC</td>
<td>10 Controls</td>
<td>Se: 100% Sp: 100%</td>
<td>1-methyl-4-(1-methylethyl)benzene, toluene, dodecane, 3,3-dimethylpentane, 2,3,4-trimethyl hexane, 1,1'-((1-butene)bis benzene</td>
<td></td>
</tr>
<tr>
<td>Peng et al. (2010)</td>
<td>SPME-GC-MS</td>
<td>40 NSCL 38 non-smokers</td>
<td>Acc: 90%</td>
<td>C₅-C₉ aldehydes</td>
<td>No influence smoking, age</td>
<td></td>
</tr>
<tr>
<td>Song et al. (2010)</td>
<td>SPME-GC-MS</td>
<td>43 NSCL 41 HC</td>
<td>Se: 95.3-93% Sp:85.4-92.7%</td>
<td>1-butanol, 3-hydroxy-2-butanone</td>
<td>C₁₁-C₁₀ aldehydes</td>
<td>Stage independent</td>
</tr>
<tr>
<td>Fuchs et al. (2010)</td>
<td>SPME-GC-MS</td>
<td>12 LC</td>
<td>12 Smokers 12 non-smokers</td>
<td>Se: 75% (pentanal) Sp: 95.8%</td>
<td>Propane, isopropyl alcohol, carbon disulfide, ethyl benzene</td>
<td>No difference between SCLC and NSCLC</td>
</tr>
<tr>
<td>Rudnicka et al. (2011)</td>
<td>SPME-GC-MS</td>
<td>23 LC</td>
<td>30 HC</td>
<td>-</td>
<td>1-octene</td>
<td>LC pts were older, ethylbenzene~exogenous</td>
</tr>
<tr>
<td>Peled et al (2012)</td>
<td>SPME-GC-MS</td>
<td>53 LC</td>
<td>19 benign lung diseases</td>
<td>Acc: 88%</td>
<td>1-Octene</td>
<td></td>
</tr>
<tr>
<td>Wang et al. (2012)</td>
<td>SPME-GC-MS</td>
<td>88 LC</td>
<td>70 benign lung disease 85 HC</td>
<td>Se: 96.5% Sp: 97.5%</td>
<td>23 VOCs (abstract)</td>
<td>Stage independent</td>
</tr>
<tr>
<td>Poli et al. (2008)</td>
<td>SPME-GC-MS</td>
<td>36 NSCL</td>
<td></td>
<td></td>
<td>After resection: ↓ Isoprene, benzene ↑ Pentane, toluene, ethyl benzene</td>
<td></td>
</tr>
</tbody>
</table>
Breath Analysis
Oncology – Lung cancer

Lung Cancer → Ox stress → Inflammation

Alcohol dehydrogenase

alcohol

CYP-enzymes

Degradation

Fig. 1. Free radical-mediated lipid peroxidation: possible reactions and reaction products.
Breath Analysis
Oncology – Lung cancer

- Mutations

- 32 Adenocarcinoma, 10 squamous cell, 8 small cell
- 39 healthy controls

- n-dodecane

Handa et al. Plos One, 2014
Breath Analysis
Oncology – Lung cancer

• Mutations
  • 30 benign nodules
  • 89 lung cancer patients
    • 16 early stage, 73 advanced stage
    • 19 EGFR(+), 34 wild-type

<table>
<thead>
<tr>
<th>Group</th>
<th>Acc</th>
<th>Sens</th>
<th>Spec</th>
<th>PPV</th>
<th>NPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Early Lung Cancer vs. Benign Nodules</td>
<td>87%</td>
<td>75%</td>
<td>93%</td>
<td>88%</td>
<td>88%</td>
</tr>
<tr>
<td>EGFR mutation vs. wild type</td>
<td>83%</td>
<td>79%</td>
<td>85%</td>
<td>75%</td>
<td>88%</td>
</tr>
</tbody>
</table>

Shlomi et al. J Thorac Oncol, 2017
Breath Analysis
Oncology – Lung cancer

• Treatment
  • Surgery
    • 15 patients with suspicion for lung cancer were resected
    • 11 had early-stage lung cancer, 4 were benign
    • Breath sample at baseline and 3 weeks after resection

• 5 VOCs reduced after surgery
• Sens 100%
• Spec: 80%

Breath Analysis
Oncology – pleural mesothelioma

• Participants:
  • 21 healthy non asbestos-exposed individuals (HC)
  • 22 healthy occupational asbestos-exposed individuals (AEx)
  • 23 MPM patients (treatment-naive)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Outcome (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity</td>
<td>0.87 (0.69 – 0.97)</td>
</tr>
<tr>
<td>Specificity</td>
<td>0.70 (0.55 – 0.82)</td>
</tr>
<tr>
<td>PPV</td>
<td>0.61 (0.43 – 0.76)</td>
</tr>
<tr>
<td>NPV</td>
<td>0.91 (0.77 – 0.98)</td>
</tr>
<tr>
<td>Accuracy</td>
<td>0.76 (0.64 – 0.98)</td>
</tr>
<tr>
<td>$\text{AUC}_{\text{ROC}}$</td>
<td>0.81 (0.69 – 0.91)</td>
</tr>
</tbody>
</table>

$\text{VOCs: } P1, P3, P5, P30, P50, P54, 7P1$
Breath Analysis
Oncology – pleural mesothelioma

• Participants:
  • 52 healthy non asbestos-exposed individuals (HC)
  • 59 healthy occupational asbestos-exposed individuals (AEx)
  • 41 patients with benign asbestos-related diseases (ARD)
  • 70 patients with benign non-asbestos-related diseases (BLD)
  • 56 primary lung cancer patients (LC)
  • 52 MPM patients (treatment-naive)

Breath Analysis
Oncology – pleural mesothelioma

<table>
<thead>
<tr>
<th></th>
<th>MPM vs AEx+ARD</th>
<th>MPM vs LC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity</td>
<td>94.2% (85.1%-98.5%)</td>
<td>73.1% (59.9%-83.8%)</td>
</tr>
<tr>
<td>Specificity</td>
<td>80.0% (71.3%-87.0%)</td>
<td>71.4% (58.7%-82.1%)</td>
</tr>
<tr>
<td>PPV</td>
<td>71.0% (59.6%-80.8%)</td>
<td>70.4% (57.3%-81.4%)</td>
</tr>
<tr>
<td>NPV</td>
<td>96.4% (90.5%-99.1%)</td>
<td>74.1% (61.2%-84.4%)</td>
</tr>
<tr>
<td>Accuracy</td>
<td>84.9% (78.5%-89.9%)</td>
<td>72.2% (63.3%-80.0%)</td>
</tr>
</tbody>
</table>

VOCs (>50% of times selected):

AEx: asymptomatic former asbestos-exposed controls. ARD: patients with benign asbestos related diseases. AUC\textsubscript{ROC}: area under the receiver operator characteristic curve. MPM: malignant pleural mesothelioma. NPV: negative predictive value. PPV: positive predictive value. VOC: volatile organic compound.
Breath Analysis
Oncology – pleural mesothelioma

AEx: asymptomatic former asbestos-exposed controls. ARD: patients with benign asbestos-related diseases. AUC_{ROC}: area under the receiver operator characteristic curve. NPV: negative predictive value. PPV: positive predictive value. VOC: volatile organic compound.
Breath Analysis
Oncology – pleural mesothelioma

• Participants

<table>
<thead>
<tr>
<th></th>
<th>MPM vs AEx+ARD</th>
<th>AEx vs ARD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity</td>
<td>100% (80.7%-100%)</td>
<td>60.0% (34.6%-81.9%)</td>
</tr>
<tr>
<td>Specificity</td>
<td>91.2% (77.9%-97.7%)</td>
<td>42.1% (21.9%-64.6%)</td>
</tr>
<tr>
<td>PPV</td>
<td>82.4% (59.2%-95.3%)</td>
<td>45.0% (24.7%-66.7%)</td>
</tr>
<tr>
<td>NPV</td>
<td>100% (90.8%-100%)</td>
<td>57.1% (31.2%-80.4%)</td>
</tr>
<tr>
<td>Accuracy</td>
<td>93.8% (84.0%-98.4%)</td>
<td>50.0% (33.6%-66.4%)</td>
</tr>
<tr>
<td>AUC&lt;sub&gt;ROC&lt;/sub&gt;</td>
<td>0.943 (0.866-1.000)</td>
<td>0.365 (0.435-0.818)</td>
</tr>
</tbody>
</table>


VOCs:
- Diethyl ether
- Limonene
- Nonanal
- Cyclohexane
- VOC I<sub>k</sub> 1287
- Isothiocyanatocyclohexane
- Hexane

Breath Analysis
Oncology – pleural mesothelioma

ROC Curve

<table>
<thead>
<tr>
<th></th>
<th>MPM vs AEx+ARD</th>
<th>AEx vs ARD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity</td>
<td>81.5% (63.7%-92.9%)</td>
<td>58.3% (30.3%-82.8%)</td>
</tr>
<tr>
<td>Specificity</td>
<td>54.5% (26.0%-81.0%)</td>
<td>46.7% (23.2%-71.3%)</td>
</tr>
<tr>
<td>PPV</td>
<td>81.5% (63.7%-92.9%)</td>
<td>46.7% (23.2%-71.3%)</td>
</tr>
<tr>
<td>NPV</td>
<td>54.5% (26.0%-81.0%)</td>
<td>58.3% (30.3%-82.8%)</td>
</tr>
<tr>
<td>Accuracy</td>
<td>73.7% (58.1%-85.8%)</td>
<td>51.9% (33.4%-70.0%)</td>
</tr>
<tr>
<td>AUC&lt;sub&gt;ROC&lt;/sub&gt;</td>
<td>0.747 (0.582-0.913)</td>
<td>0.550 (0.322-0.778)</td>
</tr>
</tbody>
</table>

AEx: asymptomatic former asbestos-exposed controls. ARD: patients with benign asbestos related diseases. AUC<sub>ROC</sub>: area under the receiver operator characteristic curve. HC: healthy controls. MPM: malignant pleural mesothelioma. NA: not applicable. NPV: negative predictive value. PPV: positive predictive value.

Urine Analysis
Oncology – colorectal cancer

- Fecal Immunochemical Testing (se: 66%-88%; sp: 87%-96%)
- Breath VOCs (37 CRC patients, 41 controls)

Acc: 85%, se: 86%, sp: 83%

VOC Analysis
Oncology – colorectal cancer

- Urine VOCs (83 CRC patients, 50 controls)

Acc: 74%, sens: 88%, spec: 60%

Arasaradnam et al, Plos One, 2014
Breath Analysis
Inflammatory bowel disease

• Crohn’s disease (CD)
• Ulcerative colitis (UC)

• Interaction between genes, environment and microbiome

• Accurate phenotyping:
  • Treatment selection
  • Endoscopy
Breath Analysis
Inflammatory bowel disease

- 25 CD patients
- 29 UC patients
- 22 Healthy Controls

Sens: 74%
Spec: 75%
AUC: 0.82

Sens: 67%
Spec: 67%
AUC: 0.70

Arasaradman et al. Dig Liver Dis, 2016
Breath Analysis
Liver disease

- 22 patients with hepatic encephalopathy (HE; 13 covert, 9 overt)
- 20 healthy controls

**Graphs:**

a) **glmnet (auc=0.84) (95% CI: 0.75, 0.93)**
   - Sens: 88% (73%-95%)
   - Spec: 68% (51%-81%)
   - HE vs HC

b) **glmnet (auc=0.71) (95% CI: 0.57, 0.84)**
   - Sens: 88% (73%-95%)
   - Spec: 68% (51%-81%)
   - Covert vs overt

Breath Analysis
Renal failure

- 86 patients with end stage renal failure
- 26 healthy controls

- Five VOCs:
  - Ammonia
  - Acetaldehyde
  - 2-propanol
  - NO$^+76$
  - O$_2^+50$

Breath Analysis
Neurological disease

- 39 patients with Alzheimer’s disease
- 16 patients with Parkinson’s disease
- 35 healthy controls
Breath Analysis
Infectious disease - tuberculosis

- *Mycobacterium tuberculosis*
- Sputum test: 62% sensitivity
- Breath:

![Graph](image)

Sens: 81%
Spec: 79%
AUC: 0.92

Sahota et al. Tuberculosis, 2016
VOC Analysis
Infectious disease – *C. difficile*

- 213 samples analysed
- 71 confirmed *C. difficile* positive microbiological evaluation

Sens: 92%
Spec: 86%
AUC: 0.93

Breath Analysis
Infectious disease – *Cystic fibrosis*

- 13 CF patients *S. aureus* +
- 5 CF patients *S. aureus* -
- Sens: 100%, spec: 80%

- Headspace mono- and coculture of *P aeruginosa* and *A fumigatus*

Future Perspectives

- Challenges: technical

### TABLE 2

<table>
<thead>
<tr>
<th>Category</th>
<th>Key Factors in Breath Collection and Handling</th>
</tr>
</thead>
<tbody>
<tr>
<td>Air sampling</td>
<td>Direct sampling</td>
</tr>
<tr>
<td></td>
<td>Reusable collection bag with thorough cleaning</td>
</tr>
<tr>
<td>Air collection device</td>
<td>Disposable collection bag</td>
</tr>
<tr>
<td></td>
<td>VOCs derived from collection device</td>
</tr>
<tr>
<td></td>
<td>Disposable collection device</td>
</tr>
<tr>
<td></td>
<td>Reusable collection device (cleaning agent VOCs)</td>
</tr>
<tr>
<td>Air collection method</td>
<td>Nasal/oral sampling</td>
</tr>
<tr>
<td></td>
<td>Tidal breathing vs forced exhalation</td>
</tr>
<tr>
<td></td>
<td>Exhalation after breath hold</td>
</tr>
<tr>
<td></td>
<td>Forced exhalation</td>
</tr>
<tr>
<td></td>
<td>Flow</td>
</tr>
<tr>
<td>Environmental influences</td>
<td>Baseline samples of environmental air</td>
</tr>
<tr>
<td></td>
<td>Wash-out period by inspiratory VOC filter</td>
</tr>
<tr>
<td>Storage</td>
<td>Direct analysis</td>
</tr>
<tr>
<td></td>
<td>Storage on sorbent tubes for prolonged stabilization of VOCs</td>
</tr>
</tbody>
</table>

A European Respiratory Society technical standard: exhaled biomarkers in lung disease

Ildiko Horváth (task force co-chair)\textsuperscript{1}, Peter J. Barnes (task force co-chair)\textsuperscript{2}, Stelios Loukides (group chair)\textsuperscript{3}, Peter J. Sterk (group chair)\textsuperscript{4}, Mariann Högman (group chair)\textsuperscript{5}, Anna-Carin Olin (group chair)\textsuperscript{6}, Anton Amann\textsuperscript{7}, Balazs Antus\textsuperscript{8}, Eugenio Baraldi\textsuperscript{9}, Andras Bikov\textsuperscript{10}, Agnes W. Boots\textsuperscript{11}, Lieuwe D. Bos\textsuperscript{12}, Paul Brinkman\textsuperscript{13}, Caterina Bucca\textsuperscript{14}, Giovanna E. Carpagnano\textsuperscript{14}, Massimo Corradi\textsuperscript{15}, Simona Cristescu\textsuperscript{16}, Johan C. de Jongste\textsuperscript{17}, Anh-Tuan Dinh-Xuan\textsuperscript{18}, Edward Dompeling\textsuperscript{19}, Niki Fens\textsuperscript{20}, Stephen Fowler\textsuperscript{21}, Jens M. Hohlfeld\textsuperscript{21,22}, Olaf Holz\textsuperscript{21}, Quirijn Jøbsis\textsuperscript{23}, Kim Van De Kant\textsuperscript{19}, Hugo H. Knobe\textsuperscript{24}, Konstantinos Kostikas\textsuperscript{25}, Lauri Lehtimäki\textsuperscript{26}, Jon Lundberg\textsuperscript{27}, Paolo Montuschi\textsuperscript{28}, Alain Van Muylen\textsuperscript{29}, Giorgio Pennazza\textsuperscript{30}, Petra Reinhold\textsuperscript{31}, Fabio L.M. Ricciardolo\textsuperscript{32}, Philippe Rosias\textsuperscript{19,39}, Marco Santonico\textsuperscript{30}, Marc P. van der Schee\textsuperscript{4}, Frederik-Jan van Schooten\textsuperscript{11}, Antonio Spanevello\textsuperscript{34}, Thomy Tonia\textsuperscript{35} and Teunis J. Vink\textsuperscript{24}

Van der Schee et al. Chest. 2015
Future Perspectives

- Challenges: environment

FACTORS THAT CAN CONTRIBUTE TO THE VOC PROFILE

- GENDER
- AGE
- SMOKING
- EXERCISE
- TIME OF DAY
- BMI
- DRUGS
- TIME SINCE EATING
- TEETH BRUSHING
Take Home messages

• VOC analysis is promising for a plethora of applications
  • Breath
  • Headspace (skin, urine, stool, cell lines, tissue)
• No single biomarker fits all
  • Focus on combinations
  • Increase number of patients
• Standardisation needed
  • Sampling
  • Underpowered clinical trials
  • Current ERS technical standards on exhaled biomarkers
• Focus on combining techniques
  • Optimal information gathering
• Statistically challenging
  • High throughput
  • ‘omics’ approach
VOC analysis in health and disease