spitäler schaffhausen

Internationales Symposium
Lung disease – what can be learned from physiology?

Exhaled breath analysis – techniques and current clinical impact

St. Gallen, 3. November 2017

PD Dr. med. Yvonne Nussbaumer, Leitende Ärztin Pneumologie
Spitäler Schaffhausen
yvonne.nussbaumer@spitaeler-sh.ch

Background

Lungs: large surface (100 m2)

Components of exhaled air:

N_2, O_2, CO_2

inert gases

water vapour

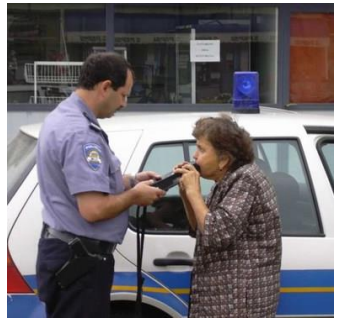
thousands of volatile and non-volatile organic components (VOC)

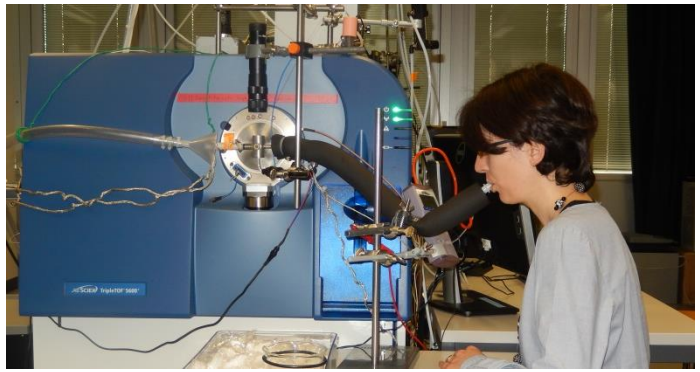
Isoprene	(12-580 ppb)
Ethanol	(13-1000 ppb)
Methanol	(160-2000 ppb)
Acetone	(1.2-1880 ppb)

….

isoprostanes, cytokines, leukotrienes, hydrogen peroxide

Background





purpose (examples)	target organ	conventional method	replacement
Ethanol (forensic)	blood	venous blood sampling	exhaled breath
Helicobacter pylori	stomach	gastric biopsy	exhaled breath (C^{13})
CO-Hb	lungs	venous blood sampling	exhaled breath

Ideal biomarker

Sensitive and specific for the diganosis of the disease

Reflect or be a very clear surrogate of the pathophysiologic mechanism

Be stable and only vary within events known to relate to disease progression

Predict early-stage disease development

Predict disease progression

Be responsive to interventions known to be effective

Sources of exhaled Nitric Oxide

Three different isoenzymes:

iNOS (NOS II)	expressed exclusively in the respiratory epithelium including the squamous epithelium of the parnyngo-oral tract
endothelial NOS (NOS III)	no detectable eNOS mRNA in lower airway epithelial cells. Expressed in endothelial cells in the respiratory tract, involved in the control of pulmonary/bronchial circulation
neuronal NOS (NOS I)	expressed in postganglionic parasympathetic neurons in the airways. inhibitory nonadrenergic noncholinergic neurotransmitter mediating bronchodilatation upon vagal stimulation.

High NO concentrations in the nasal airways in contrast to much lower levels in the lower airways (more dense iNOS expression in the epithelium of the nasal airways)

Formed non-enzymatically from nitrite (gastric ventricle, oral cavity, facultative anaerobic bacteria reduce nitrate to ntirite, vegetables (lettuce, spinach)

Relative contribution of different airway NO sources

Anatomical location	Biochemical source	Contribution to exhaled NO ppb (%)
Pharyngo-oral tract	Salivary nitrite	4 (20)
	Epithelial iNOS	4 (20)
Hypopharynx, larynx, upper trachea	Salivary nitrite/epithelial iNOS?	3 (15)
Lower trachea, bronchi	Epithelial iNOS	7 (35)
Bronchioli	Epithelial iNOS	1–2 (5–10)
Alveoli	Endothelial NOS	0.5 (2.5)

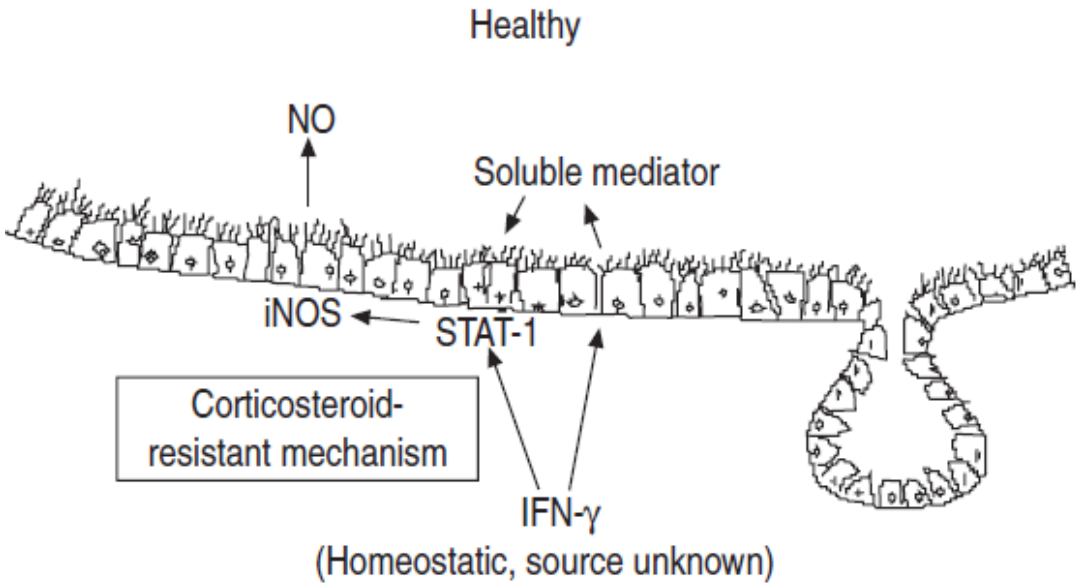
The exhaled NO concentration at a standard flow rate of 50 $mL \cdot s^{-1}$ is set to 20 parts per billion (ppb) in this subject. iNOS: inducible NO synthase; NOS: NO synthase.

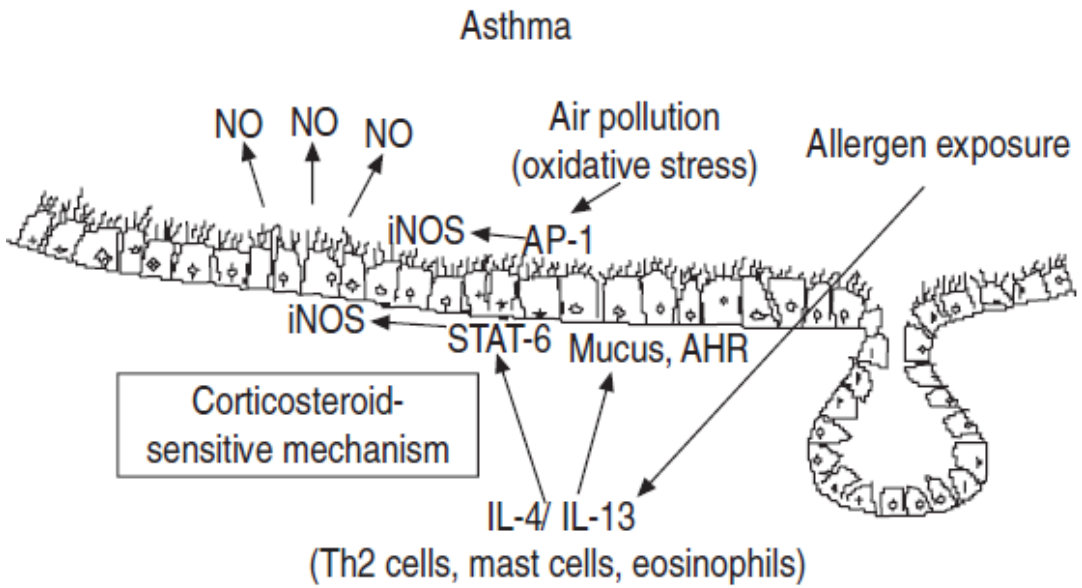
ERS Monograph: Exhaled biomarkers. Edited by I. Horvath, J.C. de Jongste. September 2010

FeNO in Asthma

Not a marker specifically of eosinophilic airway inflammation!

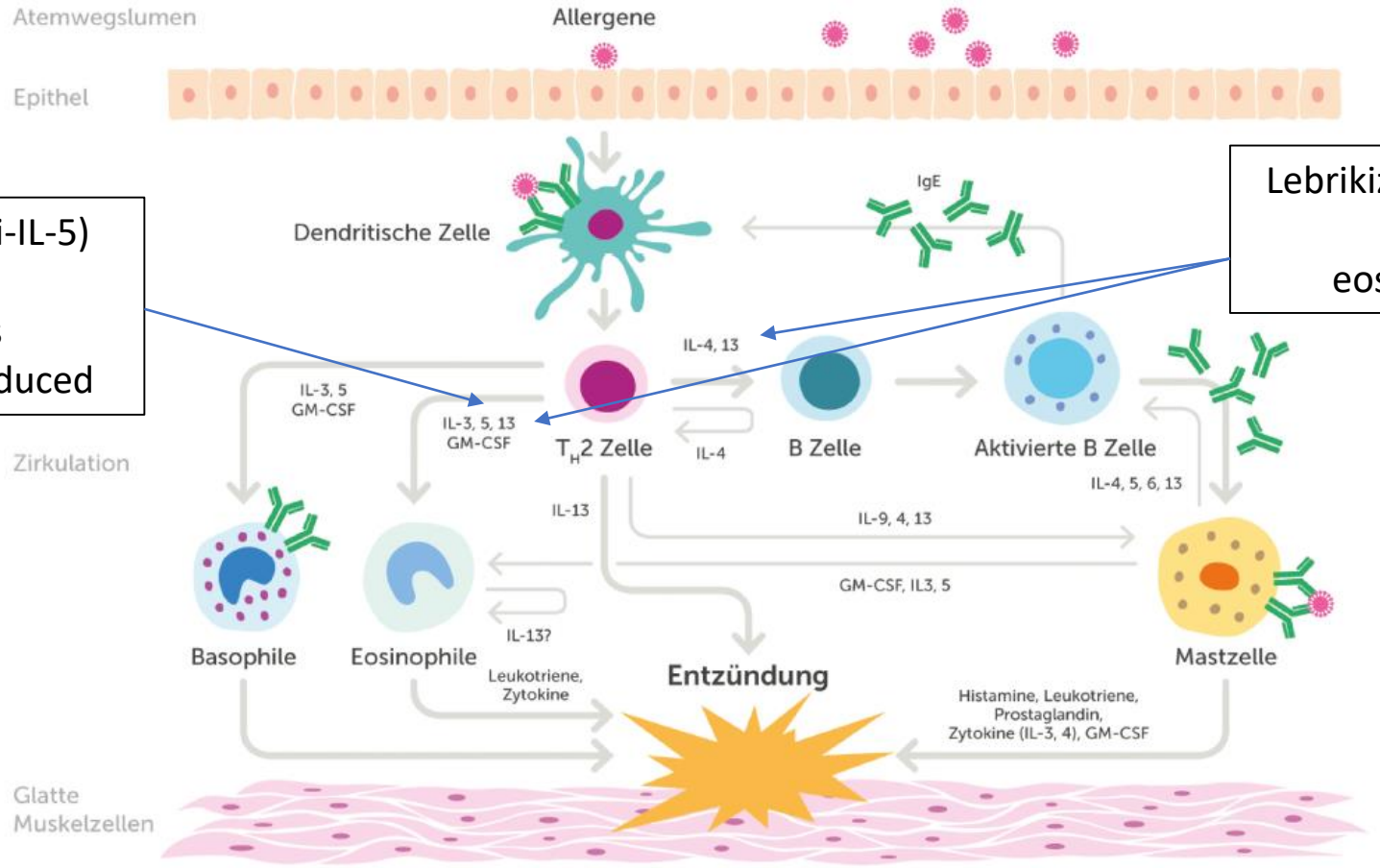
FeNO broader marker of Th2-mediated allergic inflammation (includes airway eosinophilia rather than eosinophilic inflammation only)





Mepolizumab (anti-IL-5)
FeNO idem
↓eosinophils
(sputum/blood) reduced

Lebrikizumab (anti-IL-13)
↓ FeNO
eosinophils idem



Olin J Tod, Wechsel Michael E. Asthma: pathogenesis and novel drugs for treatment. BMJ 2014;349:f5517

Factors affecting exhaled NO

Increase	Decrease
Airway infection	Smoking
Allergic rhinitis / IgE sensitisation	Exercise
Nitrate-rich diet (spinach, letucce) *	Spirometric maneuvers
Height ** / age in children (< 12 years)	Alcohol consumption
Bronchodilator	Bronchoconstriction
Diurnal variation (+ 15% afternoon)	Cystic fibrosis
	Ciliary dyskinesia
	Pulmonary hypertension

* 200 g spinach FeNO + 150%
** Increase from 120 to 180 cm associated with a doubling of FeNO.

Dweik RA, Boggs PB, Erzurum SC, et al. An official ATS clinical practice guideline: interpretation of exhaled nitric oxide levels (FENO) for clinical applications. Am J Respir Crit Care Med. 2011;184:602-615.

Taylor DR. Advances in the clinical applications of exhaled nitric oxide measurements. J Breath Res. 2012;6:047102.

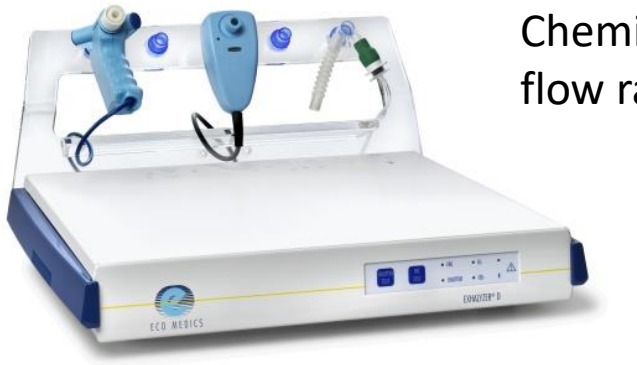
Malmberg LP, Petays T, Haahtela T, et al. Exhaled nitric oxide in healthy nonatopic school-age children: determinants and height-adjusted reference values. Pediatr Pulmonol 2006; 41: 635–642

Effect size of different patient-related factors on FeNO

Factor	Travers [230]	Malinovschi [231]	Olin [229]	Dressel [232]	Taylor [233]#
Male sex %	21 (7–39)	15 (1–31)	8 (-6–23)	17 (7–29)	25¶
Height (per 10 cm increase) %	14 (1–41)	10 (3–18)	62 (45–86)	11 (5–17)	Not significant
Current smoking %	-36 (-45– -25)	-40 (-47– -32)	-56 (-63– -50)	-39 (-42– -32)	-55¶,+
Previous smoking %	-13 (-22– -3)	-9 (-18–1)	Not significant	Not studied	Not studied
IgE sens. %	15 (0–32)	27 (15–40)	62 (45–86)	50 (37–63)§	41¶
Asthma %	17 (5–32)	46 (26–69)	41 (5–86)	Not studied separately§	26%¶

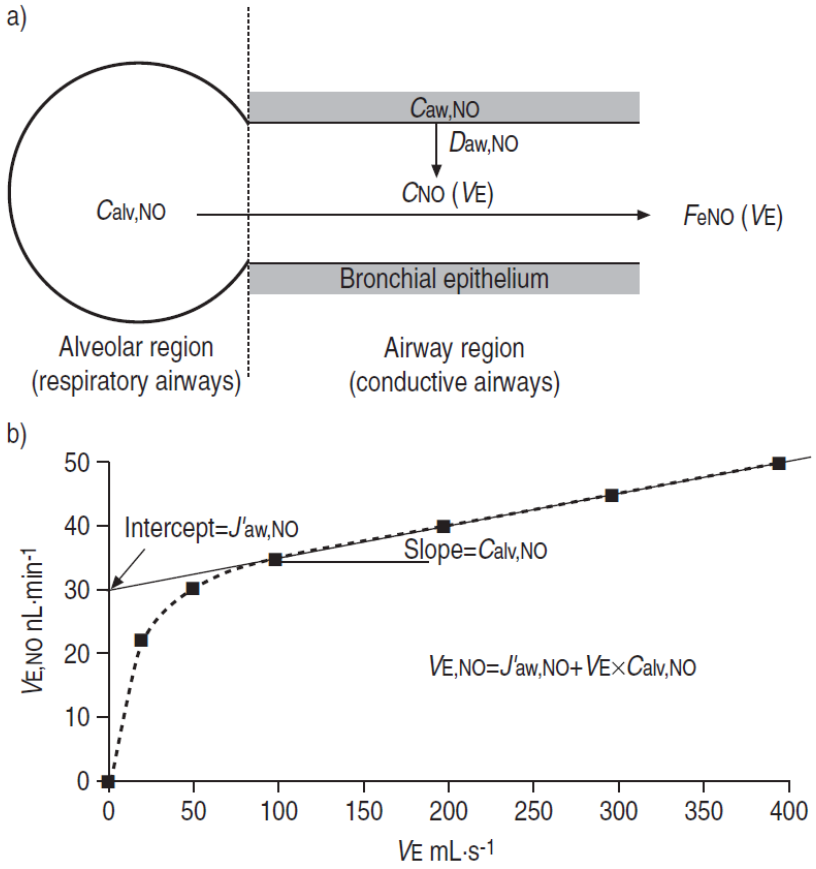
ERS Monograph: Exhaled biomarkers. Edited by I. Horvath, J.C. de Jongste. September 2010

Practical aspects of measuring exhaled NO



Chemiluminescence method
flow rate of 50 ml/s

electrochemical sensors



FeNO and its clinical impact: ICS responsiveness

ATS Guidelines 2011

We recommend the use of FeNO in determining the likelihood of steroid responsiveness in individuals with chronic respiratory symptoms possibly due to airway inflammation (strong recommendation, low quality of evidence).

Use of cut points rather than reference values

FENO (ppb)	Response to Inhaled Corticosteroids	Consider
< 25	Unlikely	Noneosinophilic asthma
< 20, children		VCD
under 12 years		Rhinosinusitis
		GERD
		Anxiety
		Cardiac disease
25-50	Possible, Cautious Interpretation	High levels of allergen exposure
20-35, children		Infection
under 12 years		
> 50[a] 39	Likely	Atopic asthma
> 35[a], children 36		High levels of allergen exposure
under 12 years		Infection
		COPD with mixed inflammatory phenotype
		Eosinophilic bronchitis

New reference values (National Health and Nutrition Examination Survey)

FeNO Interpretation in patients with asthma-like symptoms not treated with ICS

Mummadi SR et al. Update on Exhaled Nitiric Oxide in Clinical Practice. Chest 2016;149:1340-1344

FeNO and its clinical impact: Asthma Management

Conflicting results on whehter FeNO-guided management results in reduced exacerbation rates

Cochrane review 2016 (n=1700, age 28-54 years)

- asthma exacerbations significantly lower in the FeNO group compared to the control group (OR 0.60, 95% CI 0.43 to 0.84), number needed to treat to benefit over 52 weeks was 12 (95% CI 8 to 32).
- no difference between the groups for exacerbations requiring hospitalisation (OR 0.14, 95% CI 0.01 to 2.67) or rescue oral corticosteroids (OR 0.86, 95% CI 0.50 to 1.48)
- no significant difference between groups for any of the secondary outcomes (FEV1, FeNO levels, symptoms scores, or inhaled corticosteroid doses at final visit)

Authors' conclusions

… showed that tailoring asthma medications based on FeNO levels (compared with primarily on clinical symptoms) decreased the frequency of asthma exacerbations but did not impact on day-to-day clinical symptoms, end-of-study FeNO levels, or inhaled corticosteroid dose. Thus, the universal use of FeNO to help guide therapy in adults with asthma cannot be advocated. As the main benefit shown in the studies in this review was a reduction in asthma exacerbations, the intervention may be most useful in adults who have frequent exacerbations. Further RCTs encompassing different asthma severity, ethnic groups in less affluent settings, and taking into account different FeNO cutoffs are required.

Petsky H et al. Exhaled nitric oxide levels to guide treatment for adults with asthma. The Cochrane Library September 2016

Summary

FeNO testing is a noninvasive point-of-care method / indicator of Th2-mediated allergic airway inflammation

FeNO testing can assist clinicians in identifying patients with airway inflammation responsive to ICS

Incorporating FeNO testing into the management of patients with asthma may result in reductions in exacerbation rates

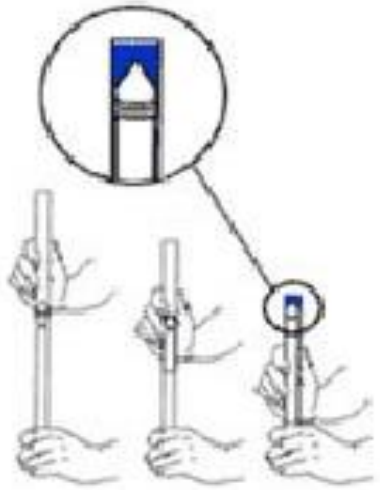
FeNO tests may have limited utility in patients with asthma phenotypes not characterized by allergic airway inflammation. Increases in FeNO levels are also seen in atopic patients without asthma.

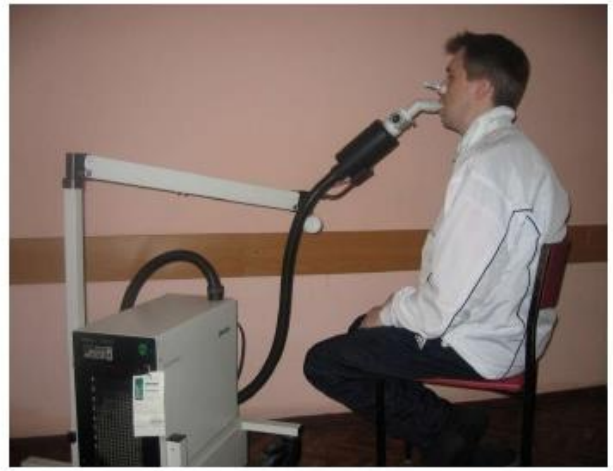
Exhaled breath condensate (EBC)

99% water vapor, small fraction of respiratory airway lining fluid droplets

Simple, safe, non-invasiv, cheap

Formed by cooling exhaled breath of patients in a condenser system





Exhaled breath condensate (EBC)

Nonvolatile biomarkers from the epithelial lining fluid of the airway epithelium

Extremly low concentrations (ultasensitive techniqes required for analysis)

pH, H_2O_2, 8-isoprostane, eicosanoids, NO, interleukins, metabolomics, proteomics, genomics …

Asthma, COPD, bronchiectasis and cystic fibrosis: elevated levels of inflammatory and oxidative stress biomarkers (hydrogen peroxide, leukotrienes, isoprostanes, hydrogen ions, prostaglandins, and nitrogen oxides).

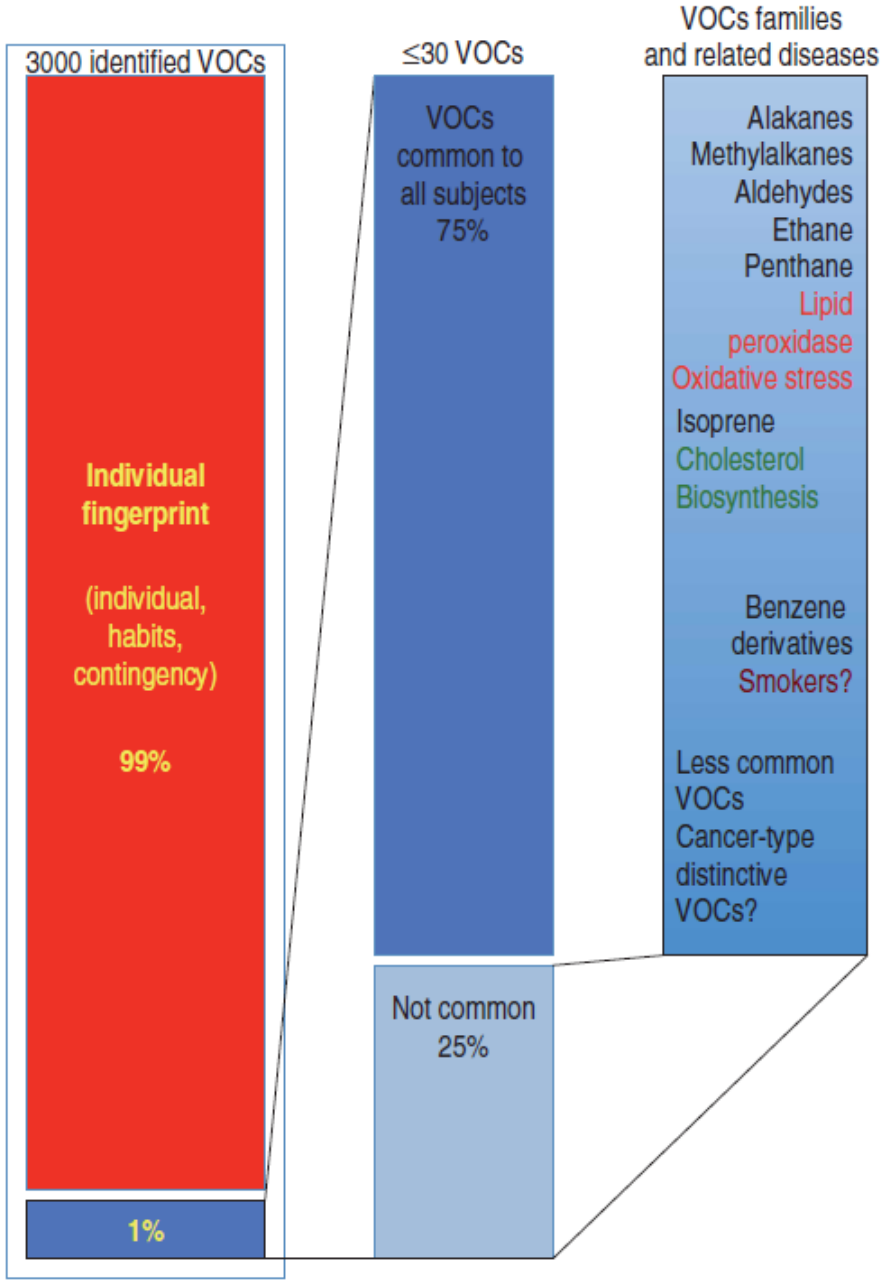
Significant difference in EBC matrix metalloproteinase 9 (MMP-9) concentrations between NSCLC and control subjects with transudative pleural effusion. Positive correlations between MMP-9 concentration and pack years smoking history ($r=0.8$, $P<0.0001$) and stage of lung cancer ($r=0.6$, $P<0.01$). No correlation with the histopathological type of lung cancer

Volatile organic compounds

Oscar the cat (NEJM 2007:357:328-329)
Steere House Nursing and Rehabiliation

Volatile organic compounds (VOC)

- Gaseous organic molecules, emitted from the fluid phase (highly volatile)
- Released from skin, with feces, urine, and breath
- Derived from many metabolic pathways.
- Rate at which VOCs are exhaled is the net effect of several interacting (bio)chemical processes; intracellular and extracellular degradation; solubility of the compound in extracellular fluid, fat, and blood; the affinity with extracellular matrix and carrier proteins; the concentration gradient with the alveolar and bronchial air; the vapor pressure; and alveolar ventilation.



Only 1% of the exhaled breath composition is likely to contain disease specific compounds

Other 99% only certain differences in compound abundance suggest disease-related information

Comorbidities?

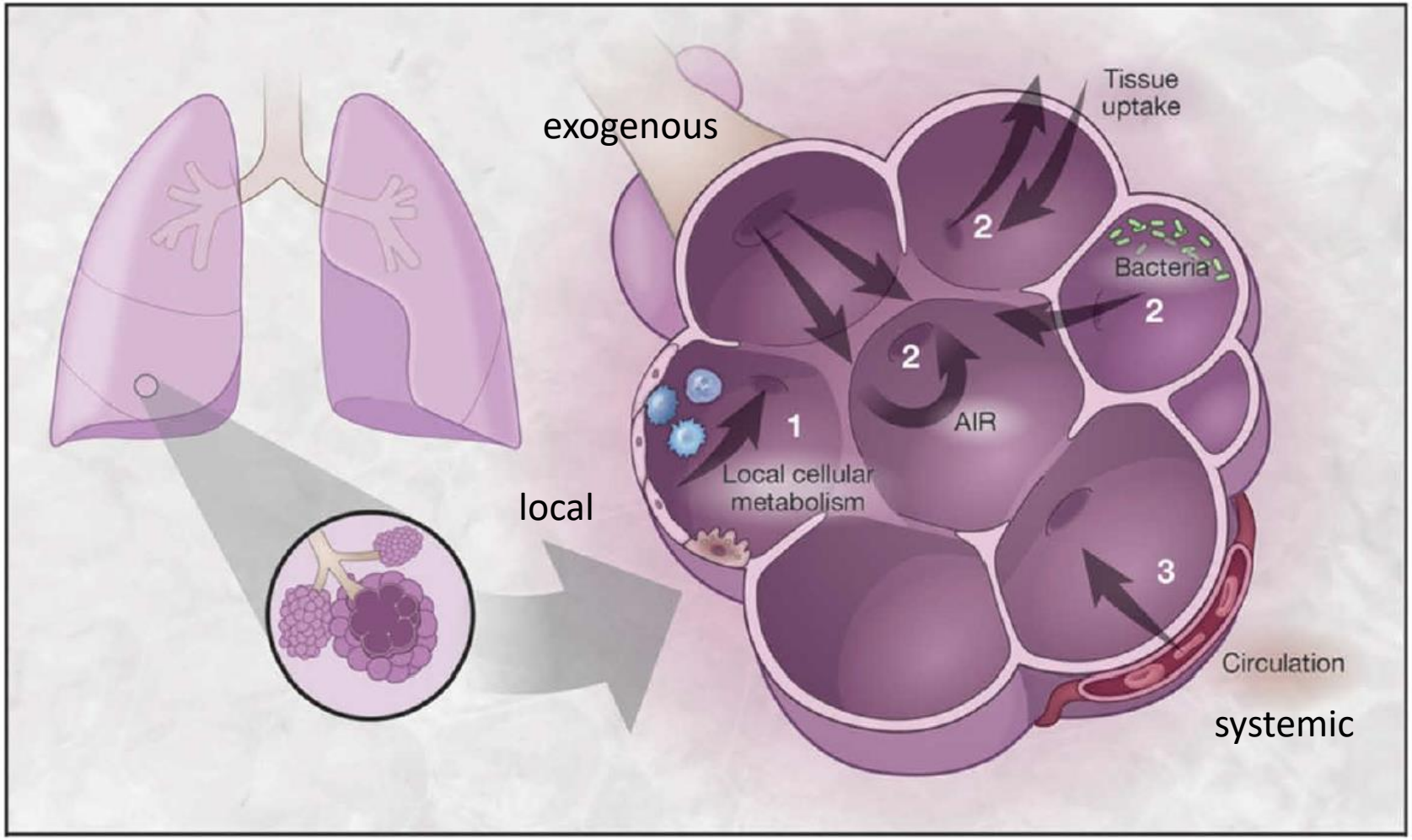
Pennazza G et al. Interpretation of exhaled volatile organic compounds. Chapter 8. ERS Monograph Exhaled Biomarkers, September 2010.

VOCs detected in lung diseases

Author	Disease	Significant VOCs identified
Phillips 2003 (13)	Lung Cancer	butane; 3-methyl tridecane; 7-methyl tridecane; 4-methyl octane; 3-methyl hexane; heptane; 2-methyl hexane, pentane; 5-methyl decane
Machado 2005 (14)	Lung Cancer	isobutane; methanol; ethanol; acetone; pentane; isoprene; isopropanol; dimethylsulfide; carbon disulfide; benzene; toluene
Poli 2005 (15)	Lung Cancer (NSCLC)	2-methyl pentane; pentane; ethyl benzene; xylenes (total); trimethyl benzene; toluene; benzene; decane; octane; penta methyl heptane
Barker 2006 (16)	Cystic Fibrosis	ethane; propane; pentane$^{*\#}$; methanol$^{\theta}$; ethanol; 2-propanol$^{\#}$; acetone; isoprene$^{\theta}$; benzene; toluene; dimethyl sulfide$^{\#\theta}$; limonene
Dragonieri 2007 (17)	Asthma	4 methyl octane; 2,4-dimethyl heptane; isopropanol; toluene; isoprene; alkane; acetic acid; acetone; 2,6,11-trimethyl dodecane; 3,7-dimethyl undecane; 2,3-dimethyl heptane
Chen 2007 (18)	Lung cancer	styrene; decane; isoprene; benzene; undecane; 1-hexene; hexanol; propyl benzene; 1,2,4-trimethyl benzene; heptanal; methyl cyclopentane
Peng 2010 (19)	Lung, breast, colon, prostate cancer	16 compounds identified that varied in abundance between healthy groups and cancer groups-1-methyl-4-(1-methyl)benzene; toluene; dodecane; 3,3-dimethyl pentane; 2,3,4-trimethyl hexane; 1,1'-(1-butenylidene) bis benzene; 1,3-dimethyl benzene;1-iodo nonane; (1,1-dimethylethyl thio) acetic acid; 4-(4-propylcyclohexyl)-4'-cyano[1,1'-biphenyl]4-yl ester benzoic acid; 2 amino-5-isopropyl-8-methyl-1-azulenecarbonitrile; 5-(2-methylpropyl) nonane; 2,3,4-trimethyl decane; 6-ethyl-3-octyl ester 2 trifluromethyl benzoic acid; p-xylene; and 2,2-dimethyldecane
Fuchs 2010 (20)	Lung cancer	Aldehydes-butanal; formaldehyde; acetaldehyde; pentanal; hexanal; octanal; nonanal
Wang 2012 (21)	Lung cancer	Adenocarcinoma-2,4,6-trimethyloctane; 2-methyldodecane; 2-tridecanone; 2-pentadecanone; 8-methy lheptadecane; 2-heptadecanone; nonadecane; eicosane; squamous-methanoic acid; 2-nonanone; 2-pentadecanone; nonadecane; eicosane; SCC-2-decanone; 2-hendecanone; 2-methylnaphthaline; 2-tridecanone; 2-pentadecanone; 2,6-dimethylnaphthaline; 1- heptadecanol; 2-heptadecanone; nonadecane; eicosane

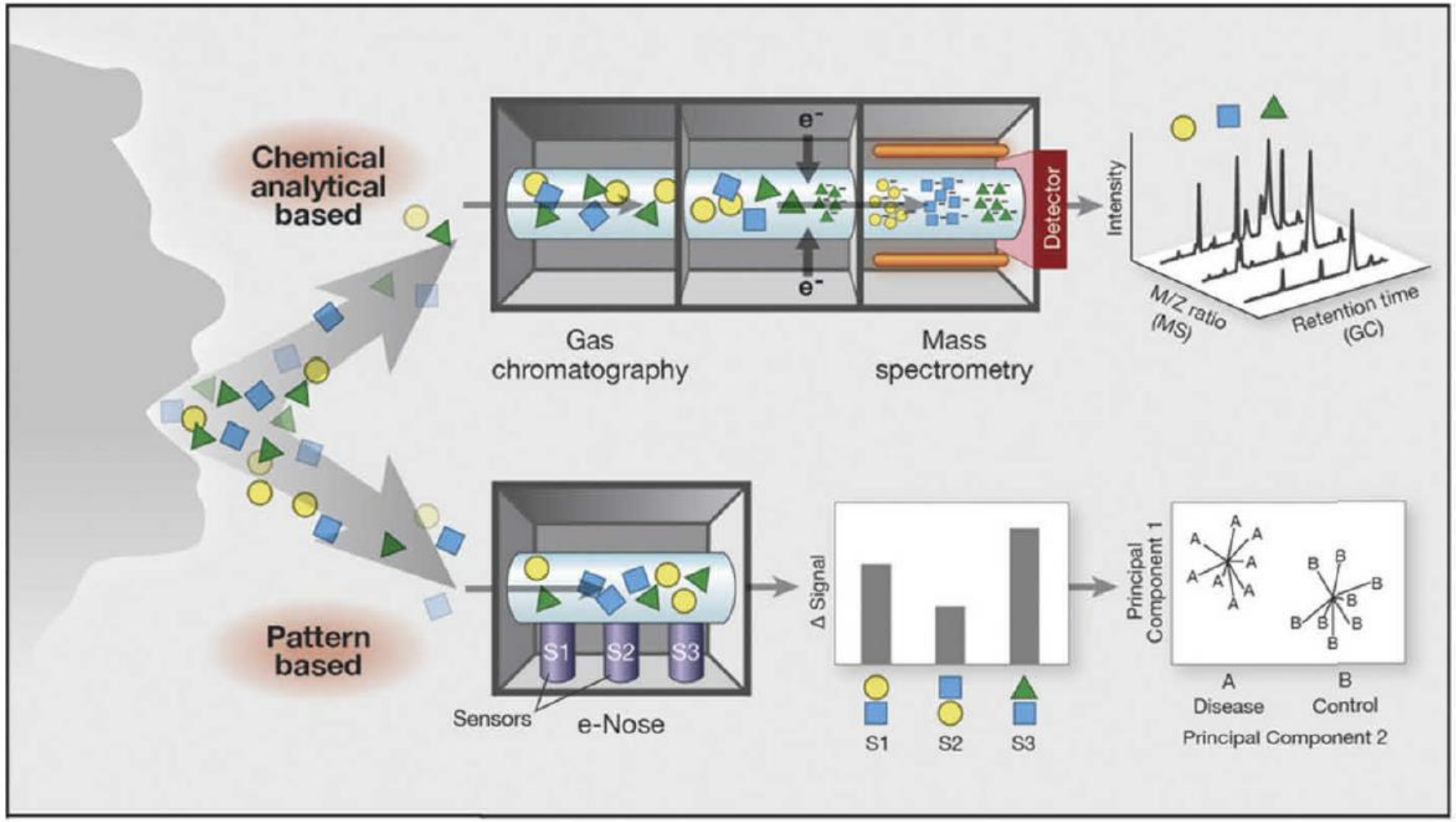
Dent AG et al. Exhaled breath analysis for lung cancer. J Thorac Dis 2013;5:S540-550

Sources of Exhaled Volatile Organic Compounds (VOC)



Van der Schee MP et al. Breathomics in Lung Disease. Chest 2015;147:224-231

Concept of Analysis of VOC



Concept of analysis of VOC

Chemical Analytical Techniques

Gas chromatography (GC) coupled to mass spectrometry (MS)

Identification of individual chemical compounds.
Clinical implementation: complex (highly trained personnel, laborious analysis)

Costly application

Recent developments:
Real-time quantitative analysis of VOCs, technically less demanding. Miniaturization of devices may allow low-cost, on-site detection of specific compounds

Pattern Recognition-Based Sensors

Electronic noses (eNoses)

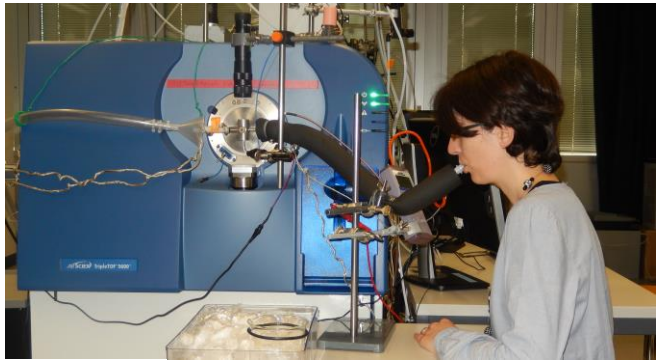
VOCs competitively interact with cross-reactive sensors, allowing multiple VOCs to interact with the same sensor based on their affinity for both the sensor and its substrate.

Does not identify individual compounds, provides probabilistic recognition, which forms the basis of assessing diagnostic accuracy.

Relatively cheap, easy to use, provides on-site results holding promise for its use as a point-of-care tool if properly validated and standardized

Exhaled breath VOC analysers

Gas chromatography and mass spectrometry (GC-MS)

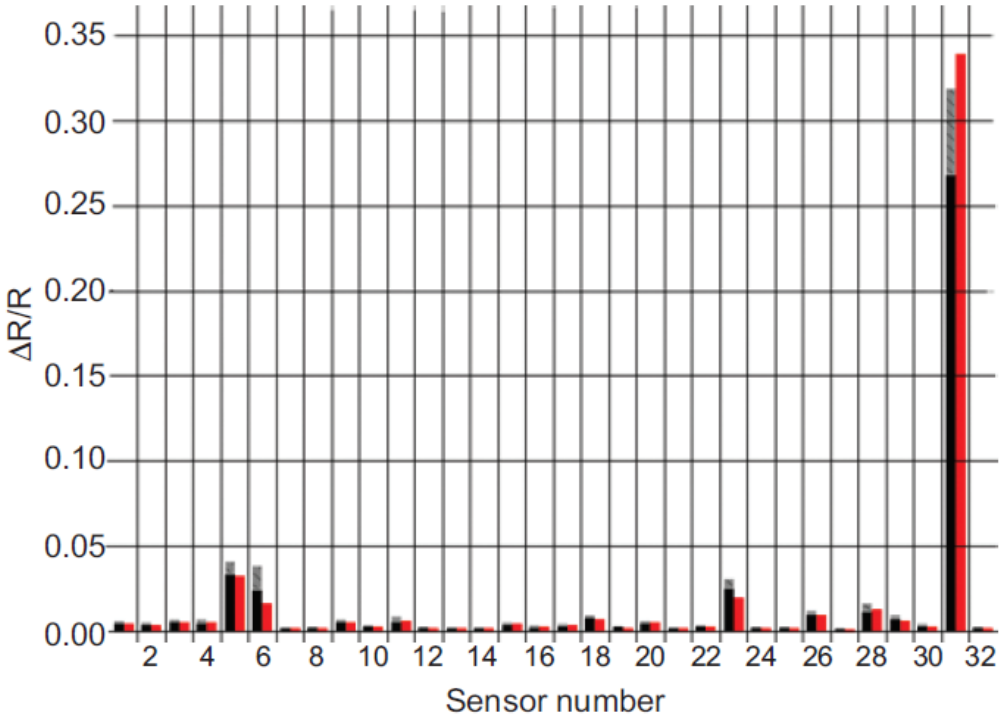


Portable/inexspensive devices

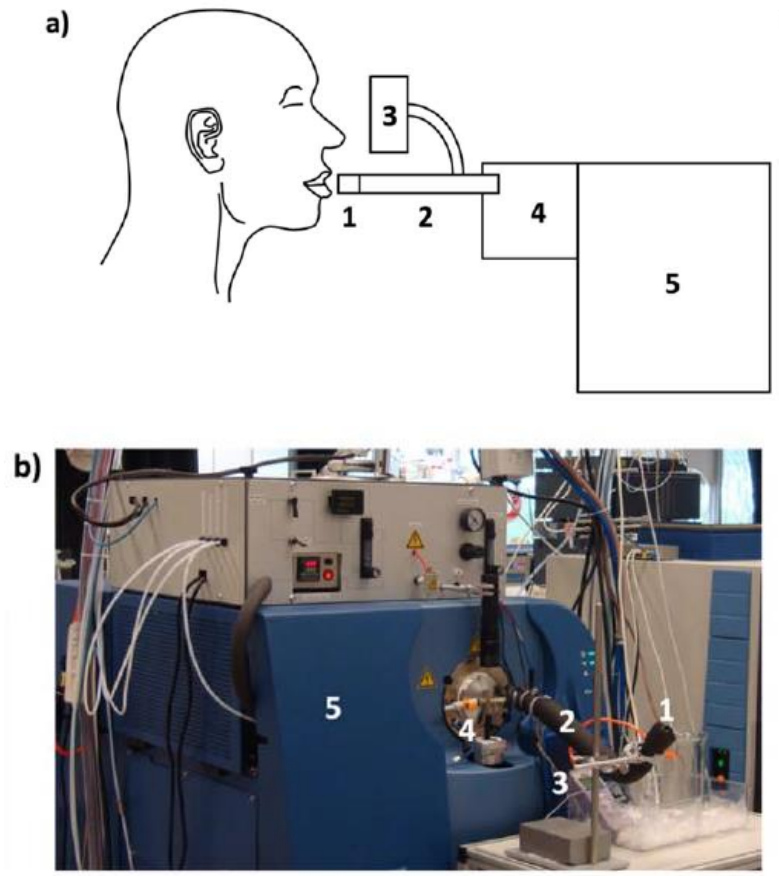
- Ion mobility spectrometry (IMS)
- Electronic noses: Cyranose 320
- Colorimetry
- Gold particle nanosensor
- Canine

Electronic nose (Cyranose 320)

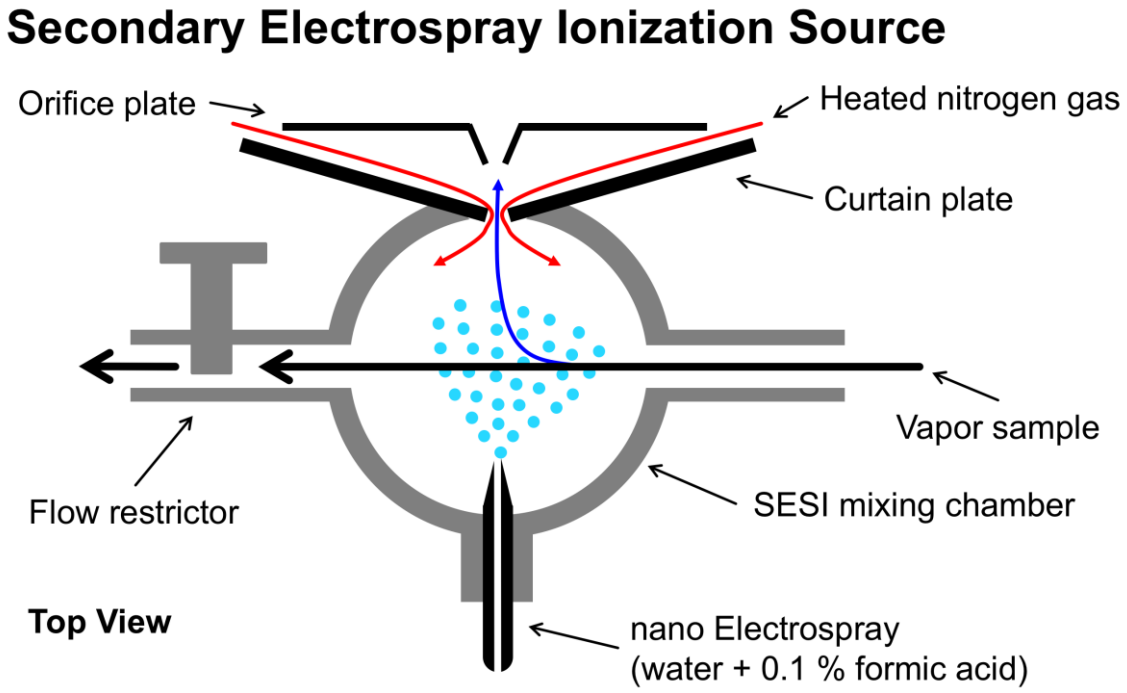
Handheld, portable
32 nanocomposite array sensors
Vapor passes over each sensor, induces a swelling and change in resistance



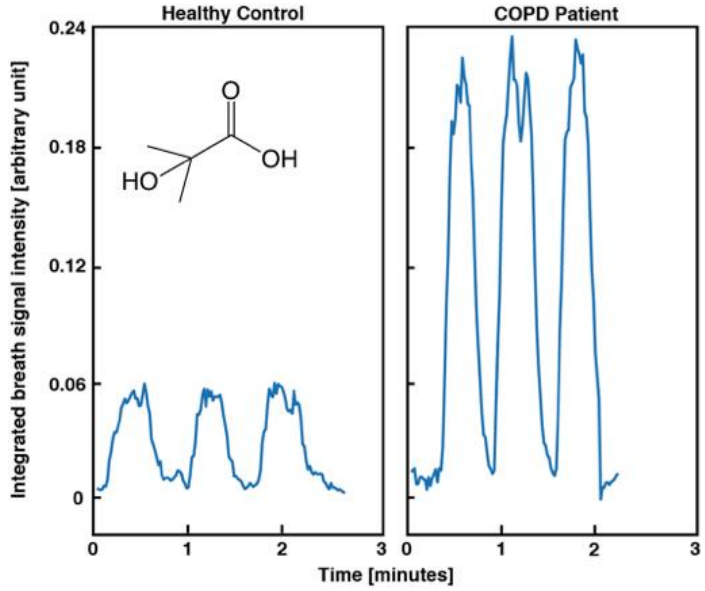
Secondary Electrospray Ionization Source (SESI)

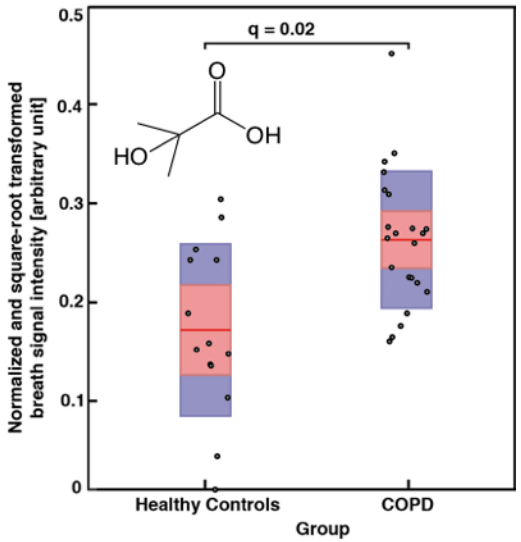


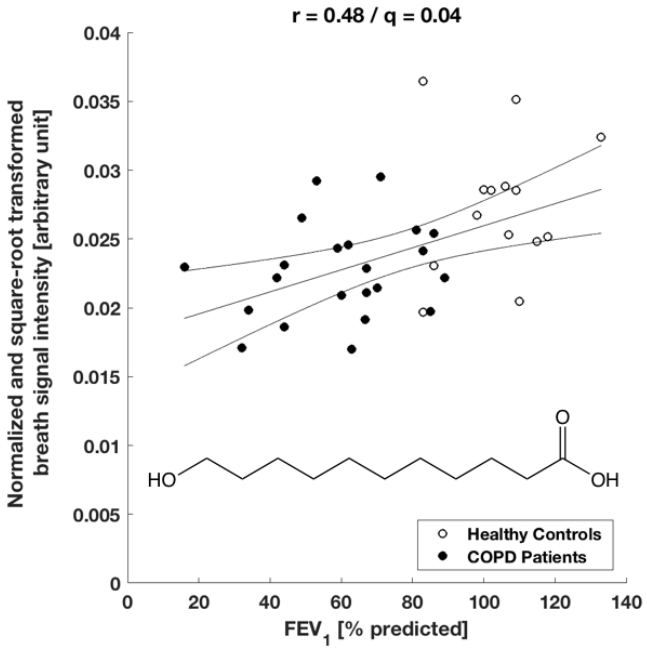
courtesy of Lukas Bregy, ETH Zurich

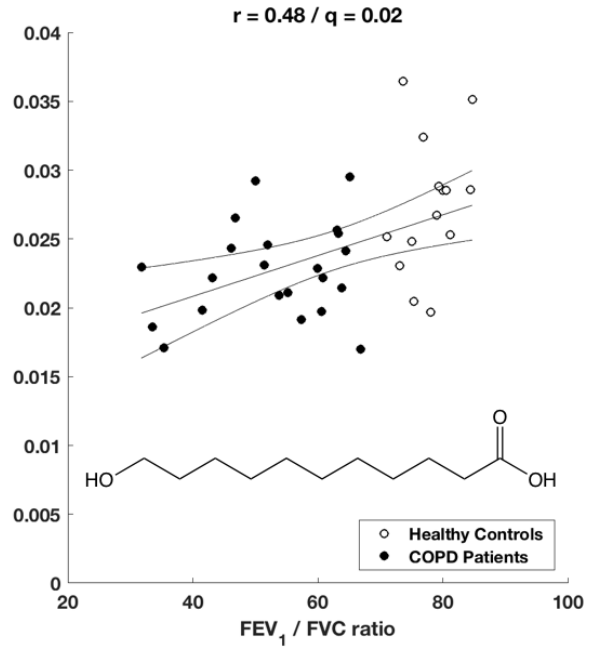


VOC in COPD: 2-hydroxyisubutyric acid



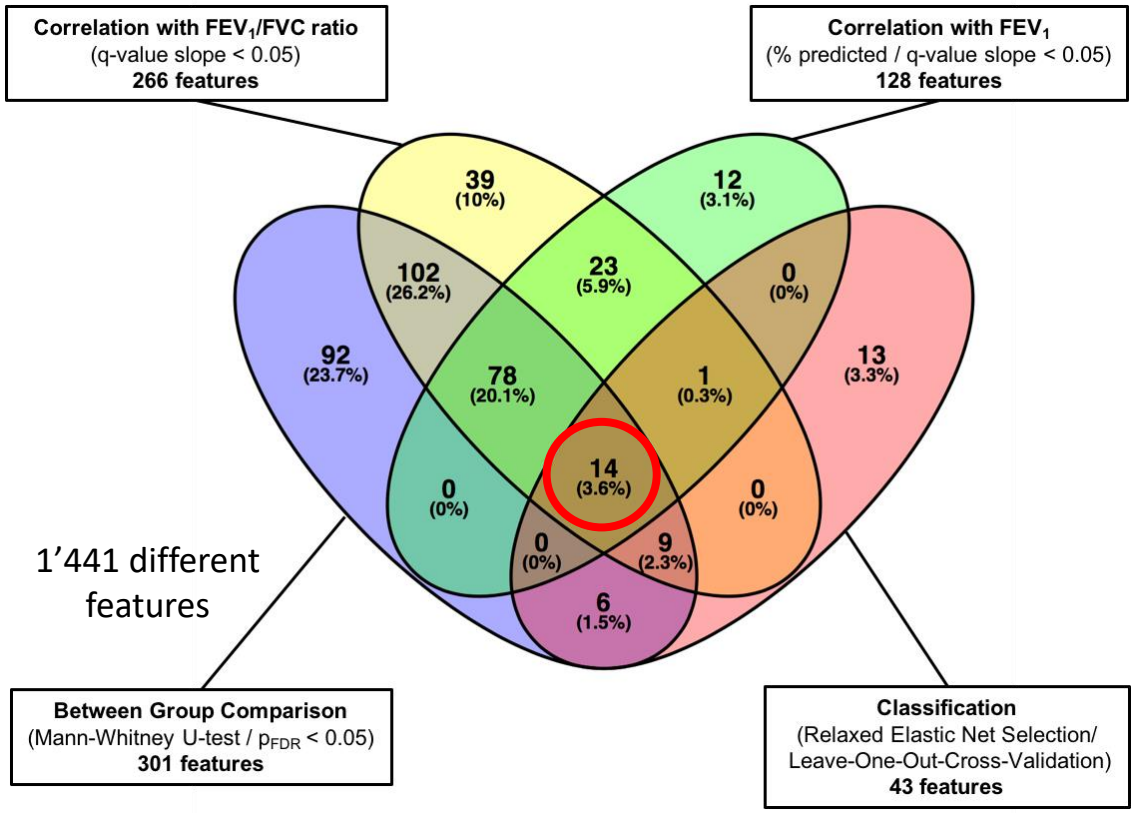






VOC in COPD

N=22 COPD (mean FEV_1 59±20%, 32±19 py, age 59±7 yrs)
N=14 controls (mean age 58±8 years, FEV_1 103±11% pred, 24±13 py, age 58±8 yrs)

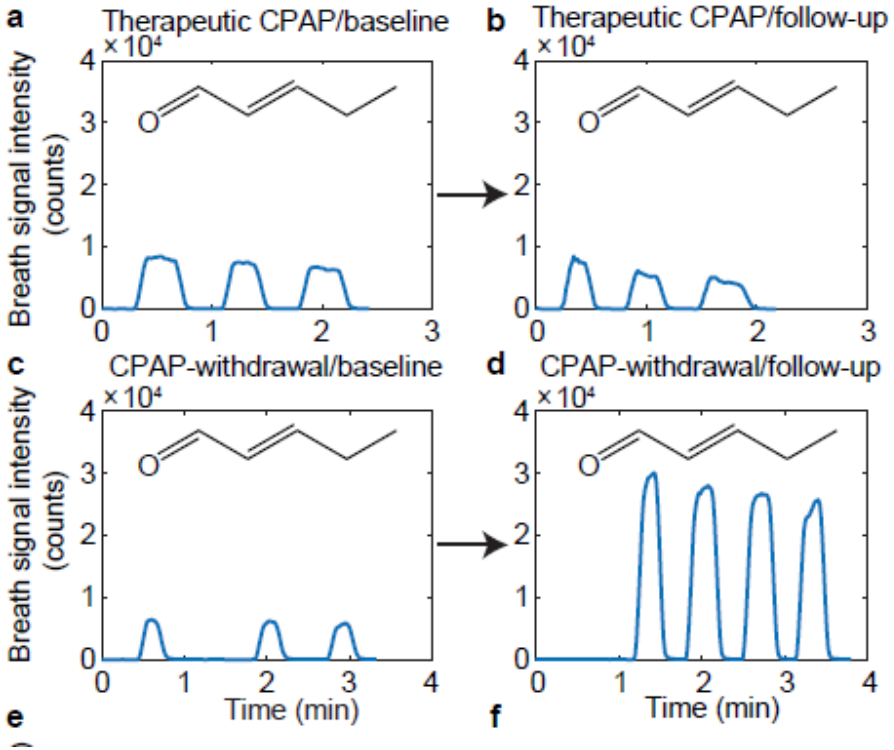


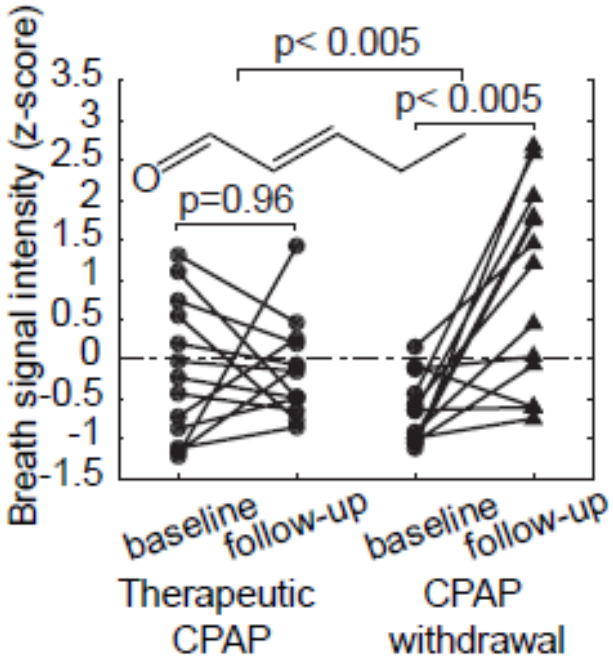
		Prediction		
Condition		COPD	No COPD	total
	COPD	19	3	22
	No COPD	1	13	14
	total	20	16	36

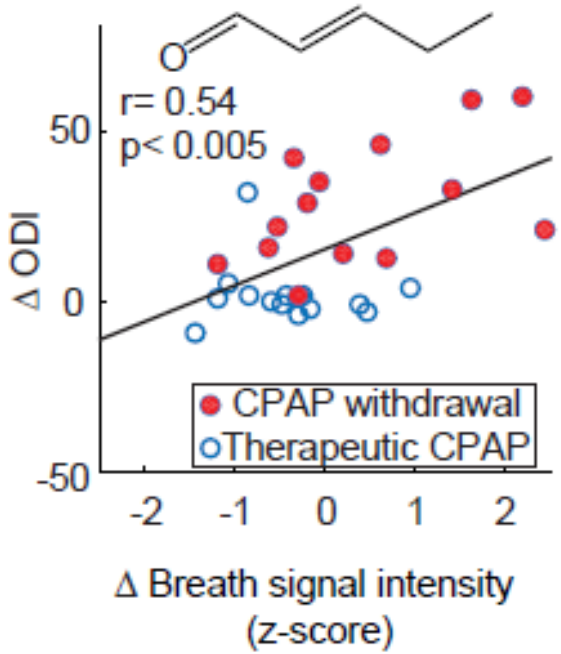
accuracy 89% (CI 74% - 97%), sensitivity 93%, specificity 87%, PPV 81%, NPV 95%

Bregy L, Nussbaumer-Ochsner Y, submitted

VOC in OSA: CPAP withdrawl







N=26 OSA patients (Δ ODI 30/h (95% CI 20/h, 40/h))

sensitivity of 92.9% and a specificity of 84.6%

Schwarz EI et al. Effects of CPAP therapy withdrawl on exhaled breath pattern in OSA. Thorax 2016;71:110-7

VOC

Real-time breath analysis by SESI-MS allows molecular profiling of exhaled breath, can distinguish patients with COPD / OSA from matched healthy controls and provides insights into the disease pathogenesis

Exact origin of most of these VOCs is unknown

- COPD: metabolites from oxidative stress processes like fatty acids, aldehydes and amino acids resulting from lung muscle degradation
- OSA: mainly aldehydes (cell membrane lipid peroxidation leads to generation of various aldehydes, e.g. isoprene (cholesterol synthesis) – sleep regulation, increased in stressful conditions such as exercise, myocardial infarction and increased cardiac output (sympathetic activity?)
- Lung fibrosis: different aminoacids (one of them proline).

Limitations: no recommended guidelines in sampling

Many variations on statistical analysis

No comparison between equipment

Confounders

Where the future goes

BreathCloud database

BreathCloud is an online reference database of exhaled biomarker profiles linked to a computer programme by a corresponding application to enable point-of-care personalized medicine. This database provides immediate diagnostic answers for the individual patient. For each patient BreathCloud will automatically select the most optimal model, based on patient characteristics and differential diagnosis, in order to create a final report. The breath analysis outcomes of each patient will eventually - after diagnosis, established according gold standard methods, - be added to the expanding database and will automatically make subsequent diagnoses more accurate.