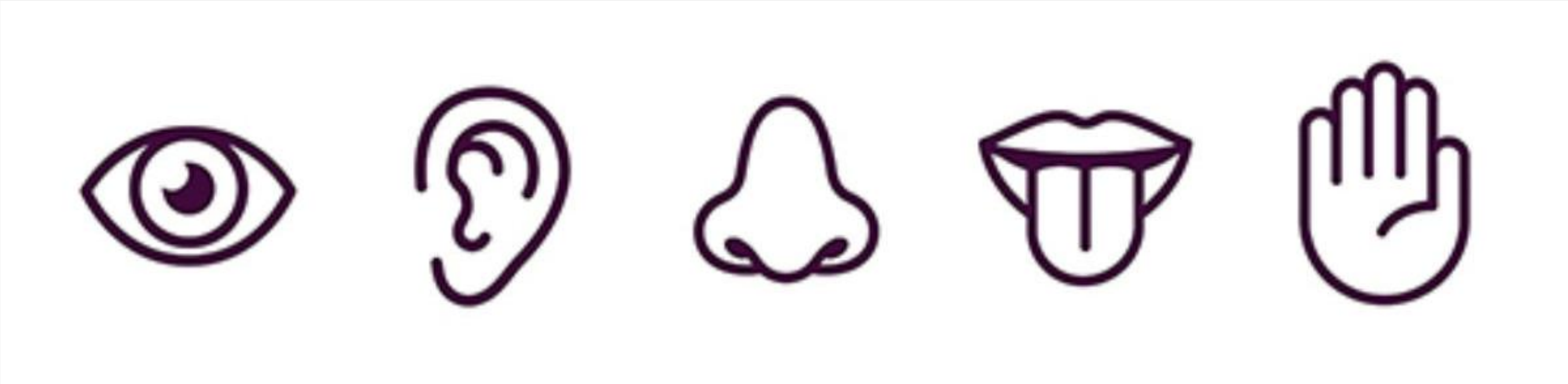
BREATH BIOMARKERS IN RESPIRATORY DISEASES

서울의대 윤호일

5 SENSES

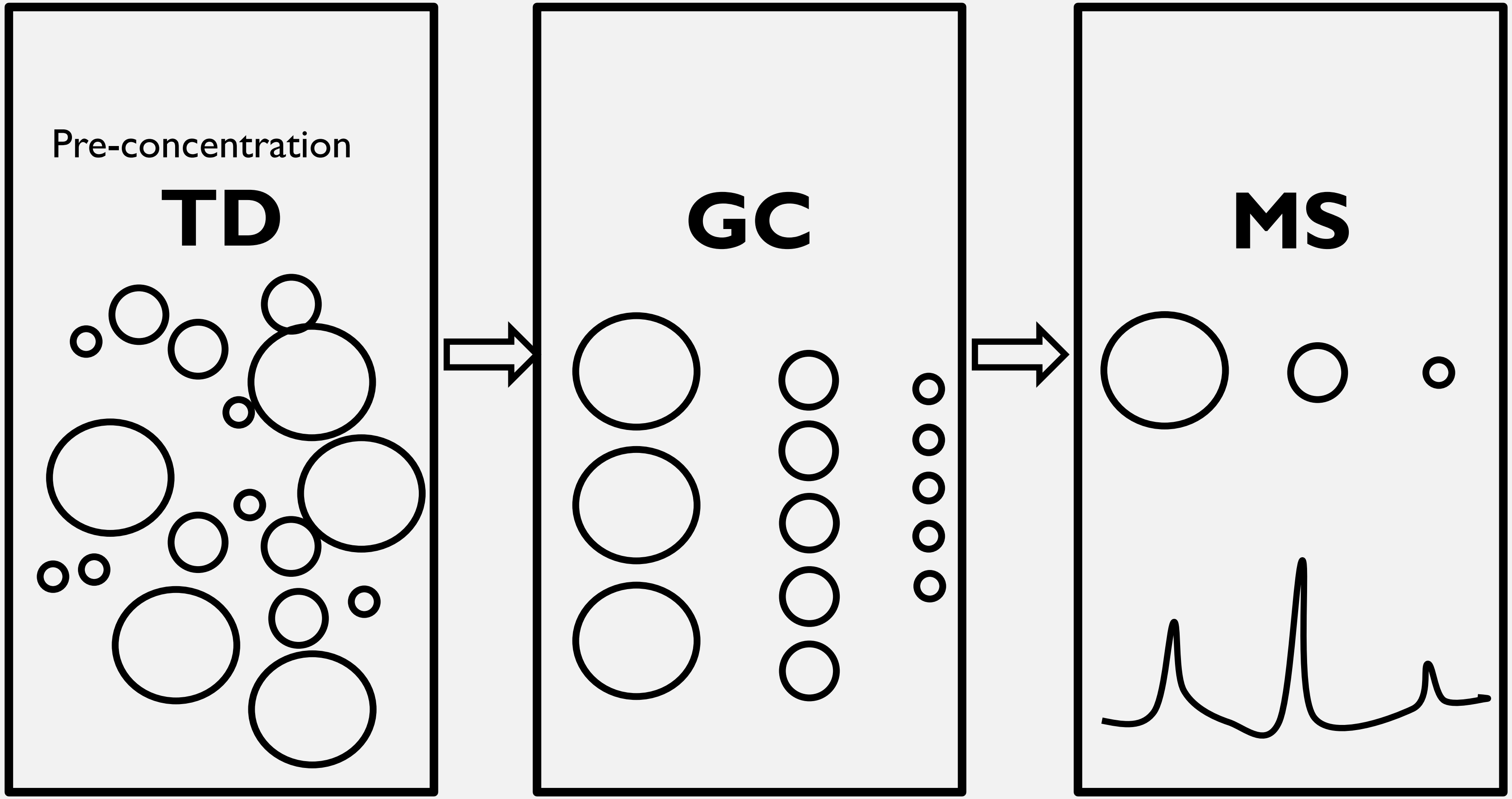
- All our knowledge begins with the senses. Emmanuel Kant (1724~1804)



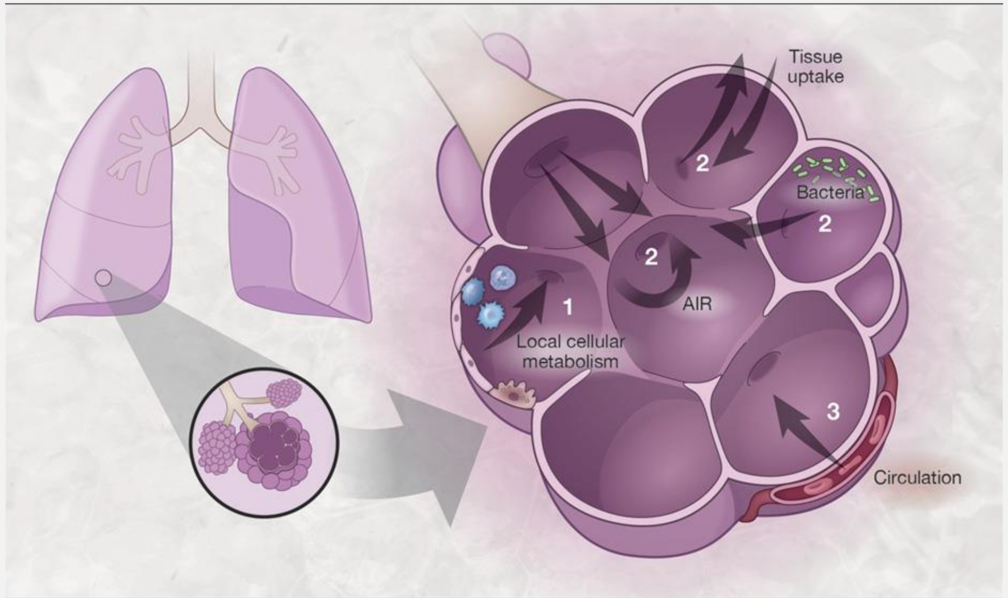
BREATH ANALYSIS

- Gas analysis
  - Spectrometric analyses (ex. GC-MS)
  - Gas sensor (ex. FeNO, electronic nose)
- Liquid analysis
  - Exhaled breath condensate (EBC)

SPECTROMETRIC ANAYLSIS



SOURCES OF VOC IN HUMAN BREATH



- Environment
- Respiratory system
- Systemic circulation

Chest 2015;247:224

WHAT'S IN MY BREATH?

%	O_2, N_2, H_2O, CO_2
ppm	$(CH_3)_2CO$, CO, CH_4, H_2, C_2H_5OH, $C_6H_{10}S$
ppb	HCHO, CH_3CHO, C_5H_{10}, C_5H_{12} C_2H_6, C_2H_4, NO, CS_2, Hydrocarbons CH_3OH, C_2H_5OH, COS, CH_3SH NH_3, CH_3NH_2, $(CH_3)_2S$

FACTORS AFFECTING EXHALED VOCS

- Environment
  - Ambient VOCs, temp, humidity, season
- Subject
  - Demographics, nutrition, smoking, medication, circadian rhythm, non-pulmonary diseases, breathing pattern
- Analysis
  - Storage, pre-concentration, collection (mixed air or alveolar air), collection method

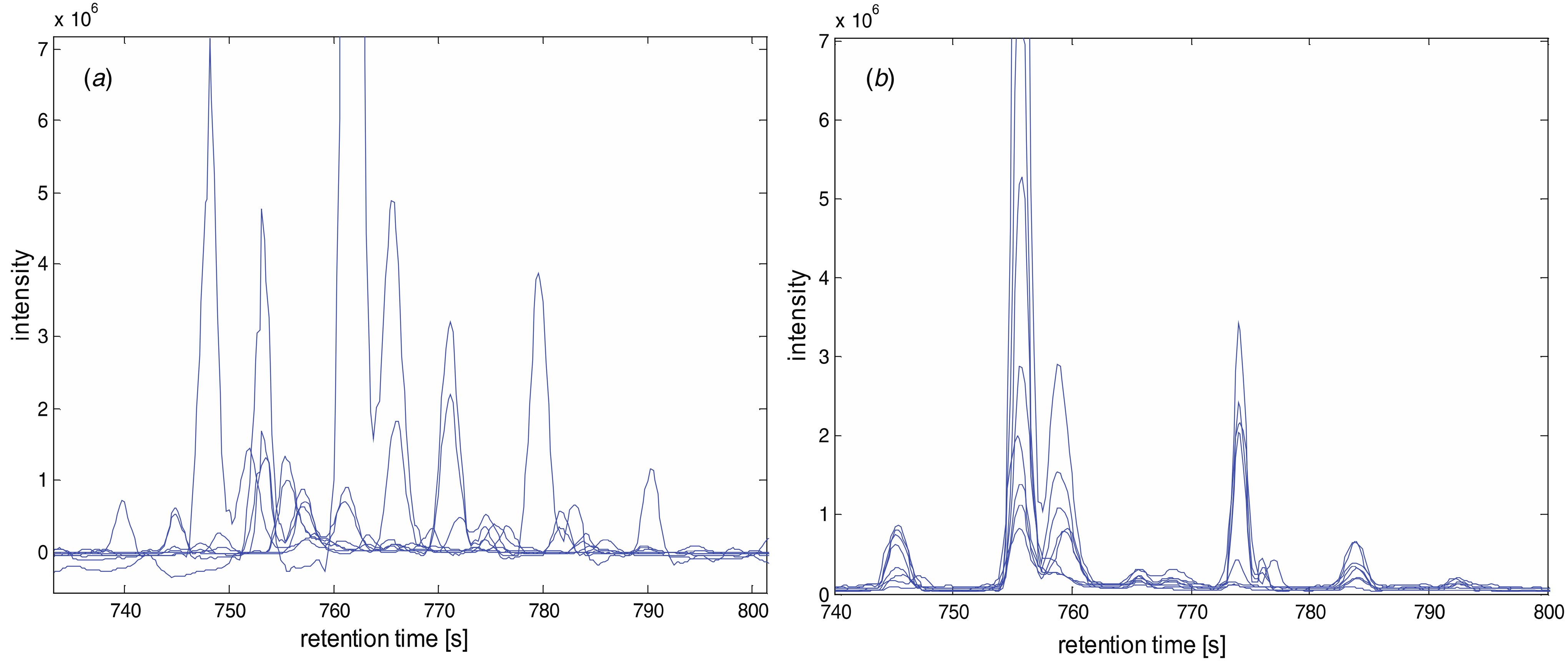
KEY FACTORS IN BREATH COLLECTION

- Air sampling
- Collection device
- Collection method
- Environmental influences
- Storage

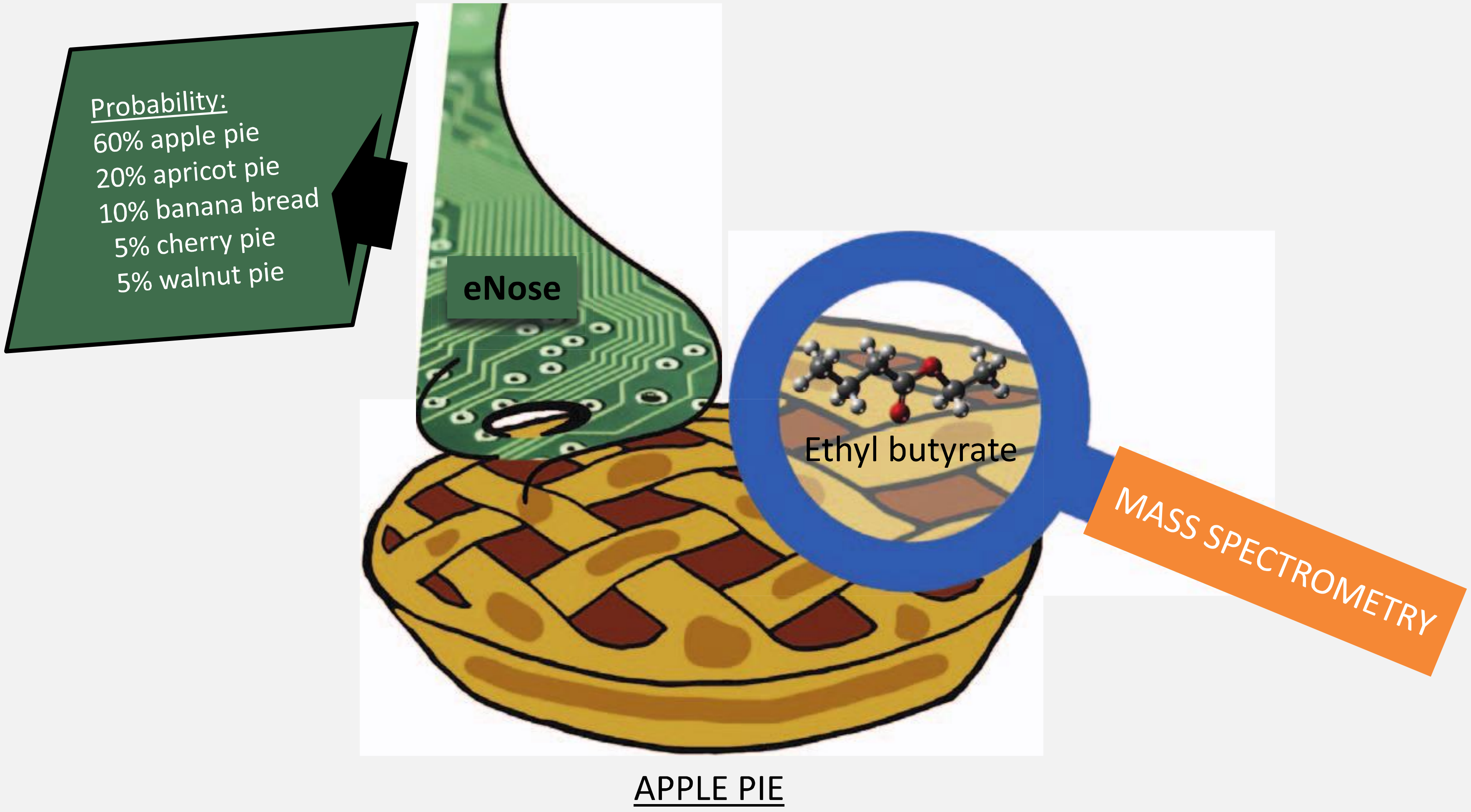
Chest 2015;247:224

BREATH® BIOPSY

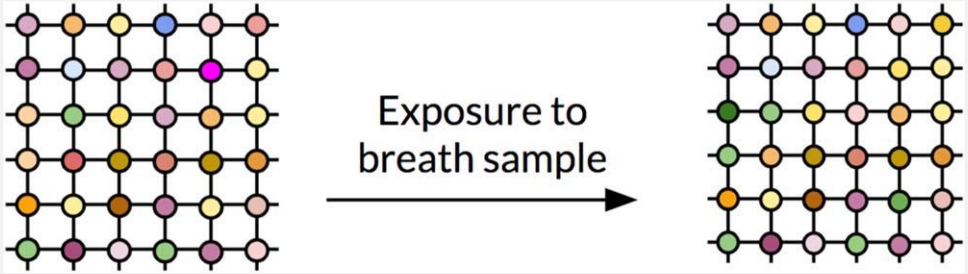
ReCIVA®
Breath Sampler

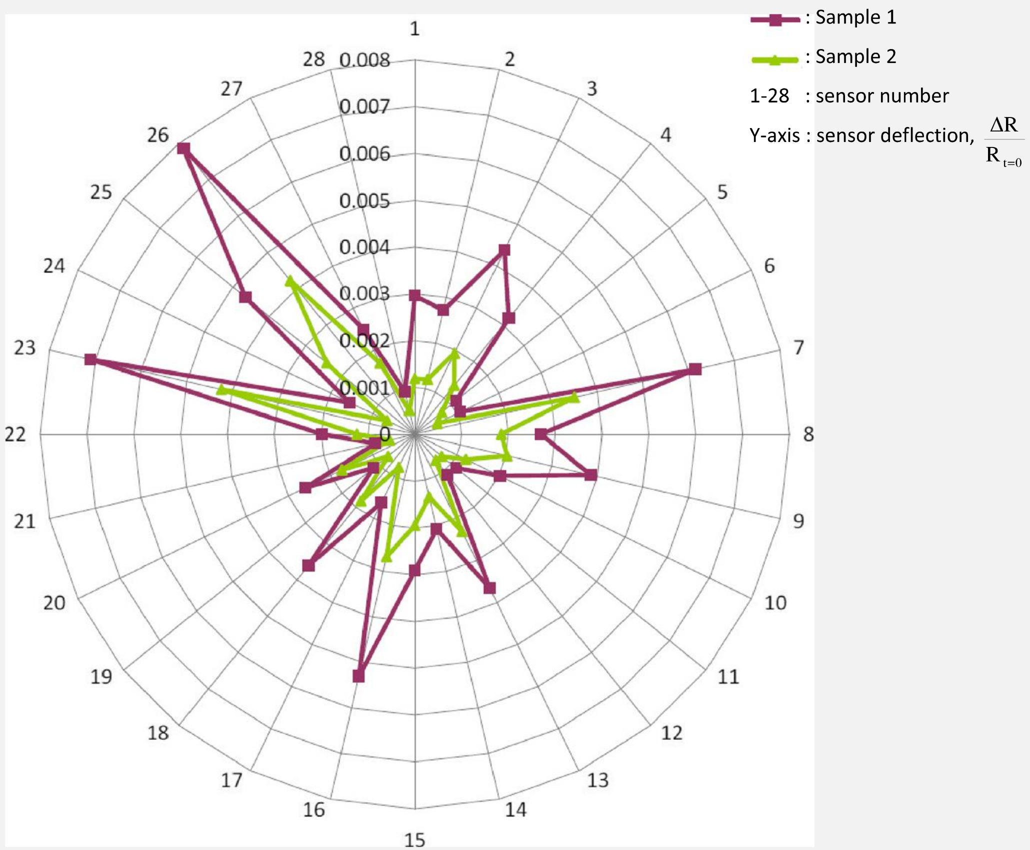


J Breath Res 2012


Probability:
60% apple pie
20% apricot pie
10% banana bread
5% cherry pie
5% walnut pie
eNose
Ethyl butyrate
MASS SPECTROMETRY
APPLE PIE

ELECTRONIC NOSE





Respir Res 2012;13:117

ACS NANO

ACS Editors' Choice

www.acsnano.org

Diagnosis and Classification of 17 Diseases from 1404 Subjects *via* Pattern Analysis of Exhaled Molecules

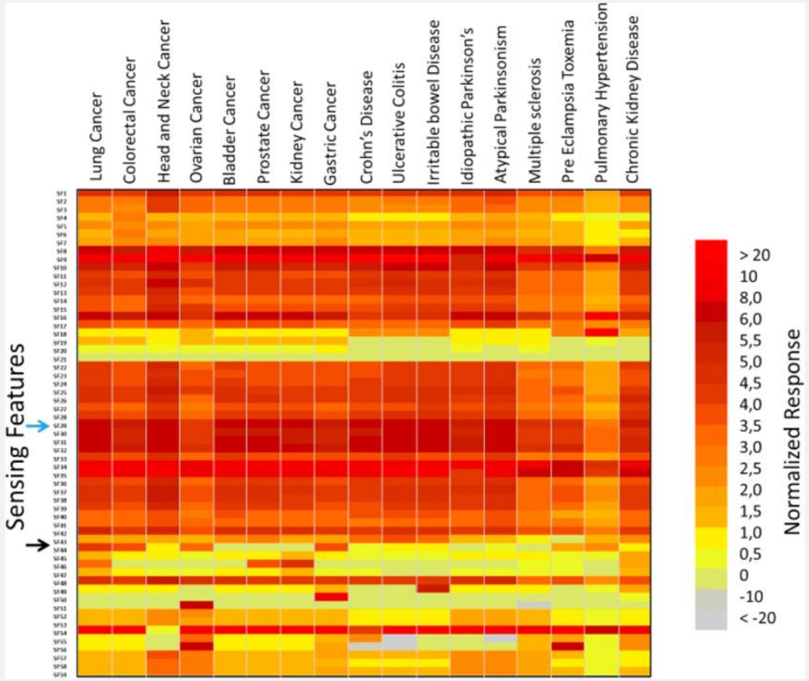
Morad K. Nakhleh,[†] Haitham Amal,[†] Raneen Jeries,[†] Yoav Y. Broza,[†] Manal Aboud,[†] Alaa Gharra,[†] Hodaya Ivgi,[†] Salam Khatib,[†] Shifaa Badarneh,[†] Lior Har-Shai,[‡] Lea Glass-Marmor,[‡] Izabella Lejbkowicz,[‡] Ariel Miller,[‡] Samih Badarny,[§] Raz Winer,[§] John Finberg,[∥] Sylvia Cohen-Kaminsky,[⊥] Frédéric Perros,[⊥] David Montani,[⊥] Barbara Girerd,[⊥] Gilles Garcia,[⊥] Gérald Simonneau,[⊥] Farid Nakhoul,[#] Shira Baram,[¶] Raed Salim,[¶] Marwan Hakim,[□] Maayan Gruber,[■] Ohad Ronen,[■] Tal Marshak,[■] Ilana Doweck,[■] Ofer Nativ,[○] Zaher Bahouth,[○] Da-you Shi,[●] Wei Zhang,[●] Qing-ling Hua,[●] Yue-yin Pan,[●] Li Tao,[●] Hu Liu,[●] Amir Karban,[△] Eduard Koifman,[△] Tova Rainis,[▲] Roberts Skapars,[▽] Armands Sivins,[▽] Guntis Ancans,[▽] Inta Liepniece-Karele,[▽] Ilze Kikuste,[▽,▼] Ieva Lasina,[▽] Ivars Tolmanis,[▼] Douglas Johnson,[⬡] [ORCID] Stuart Z. Millstone,[⬢] Jennifer Fulton,[††] John W. Wells,[⬢] Larry H. Wilf,[‡‡] Marc Humbert,[⊥] Marcis Leja,[▽,▼] Nir Peled,[§§] and Hossam Haick*[,†] [ORCID]

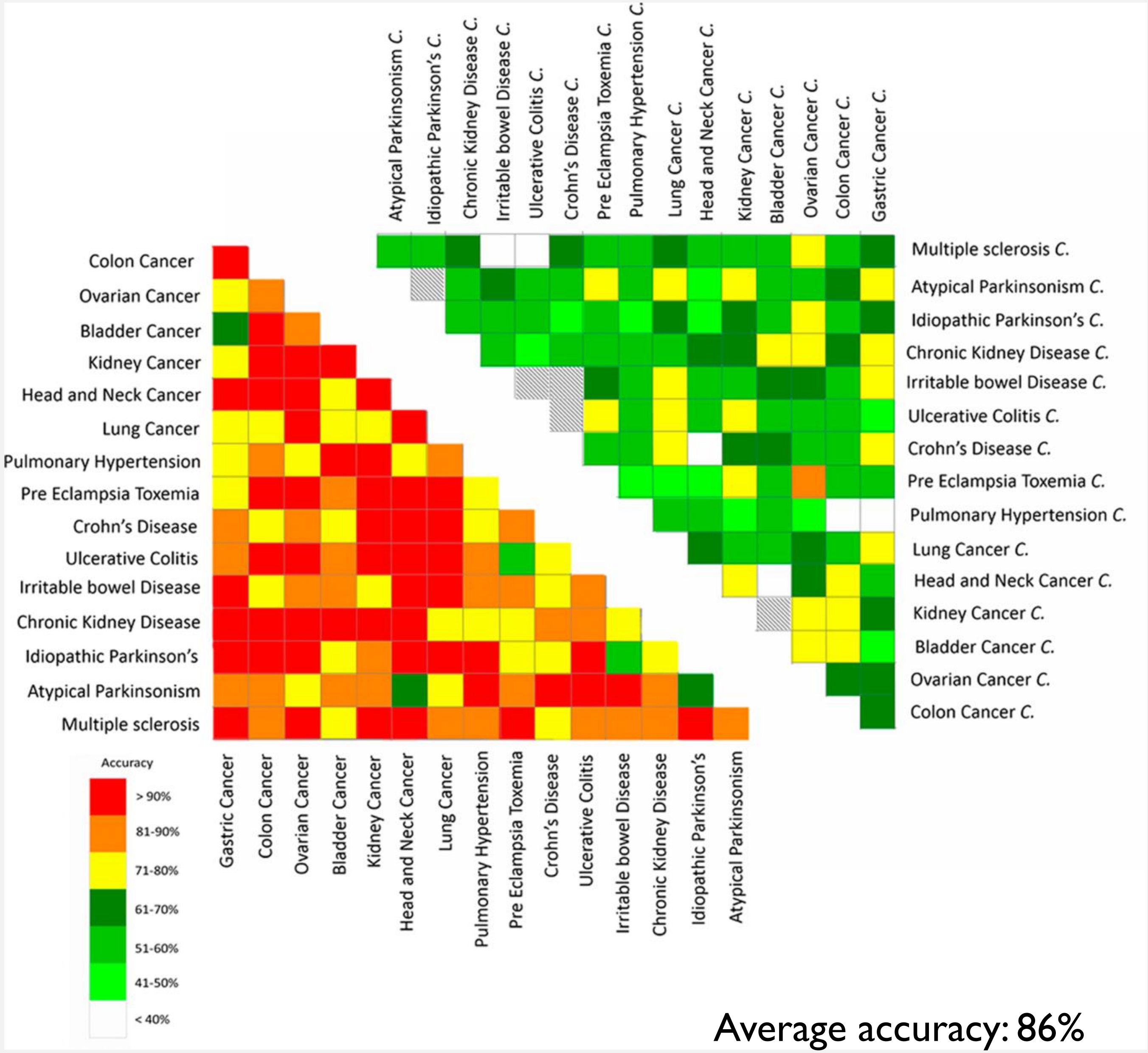
Table 1. Demographic Characteristics of Patients and Healthy Controls in the Current Study

group	patients				controls			
	n	age ± SD[a]	male, *n* (%)	smoker, *n* (%)	*n*	age ± SD[a]	male, *n* (%)	smoker, *n* (%)
lung cancer (LC)	45	67 ± 09	23 (51%)	44 (98%)	23	56 ± 14	12 (52%)	12 (52%)
colorectal cancer (CRC)	71	66 ± 10	42 (59%)	09 (11%)	89	60 ± 14	67 (75%)	09 (13%)
head and neck cancer (HNC)	22	62 ± 12	19 (86%)	13 (59%)	19	50 ± 12	06 (32%)	05 (25%)
ovarian cancer (OC)	48	51 ± 11	00 (00%)	00 (00%)	48	47 ± 09	00 (00%)	00 (0%)
bladder cancer (BC)	73	69 ± 11	68 (93%)	53 (68%)				
prostate cancer (PC)	11	66 ± 08	11(100%)	05 (45%)	35	66 ± 12	31 (88%)	25 (71%)
kidney cancer (KC)	33	65 ± 13	22 (66%)	15 (45%)				
gastric cancer (GC)	99	63 ± 12	57 (58%)	26 (27%)	155	57 ± 15	55 (34%)	23 (15%)
Crohn's disease (CD)	41	38 ± 12	23 (56%)	20 (50%)				
ulcerative colitis (UC)	37	41 ± 16	20 (56%)	16 (43%)	44	41 ± 02	28 (60%)	15 (35%)
irritable bowel syndrome (IBS)	27	38 ± 13	08 (32%)	08 (30%)				
idiopathic Parkinson's (IPD)	44	65 ± 14	23 (53%)	07 (15%)	37	62 ± 12	19 (51%)	09 (24%)
atypical Parkinsonism (PDISM)	16	67 ± 08	07 (44%)	06 (35%)				
multiple sclerosis (MS)	118	38 ± 10	42 (36%)	38 (32%)	44	39 ± 11	17 (38%)	15 (34%)
pulmonary hypertension (PAH)	22	48 ± 12	06 (27%)	12 (54%)	23	38 ± 08	10 (43%)	10 (43%)
pre-eclampsia toxemia (PET)	24	30 ± 06	00 (00%)	00 (00%)	47	29 ± 04	00 (00%)	00 (00%)
chronic kidney disease (CKD)	82	65 ± 12	52 (64%)	24 (29%)	27	46 ± 02	12 (45%)	11 (40%)
total	813	55 ± 10	423 (52%)	296 (36%)	591	52 ± 08	257 (43%)	134 (23%)

[a]Age given as mean ± standard deviation.

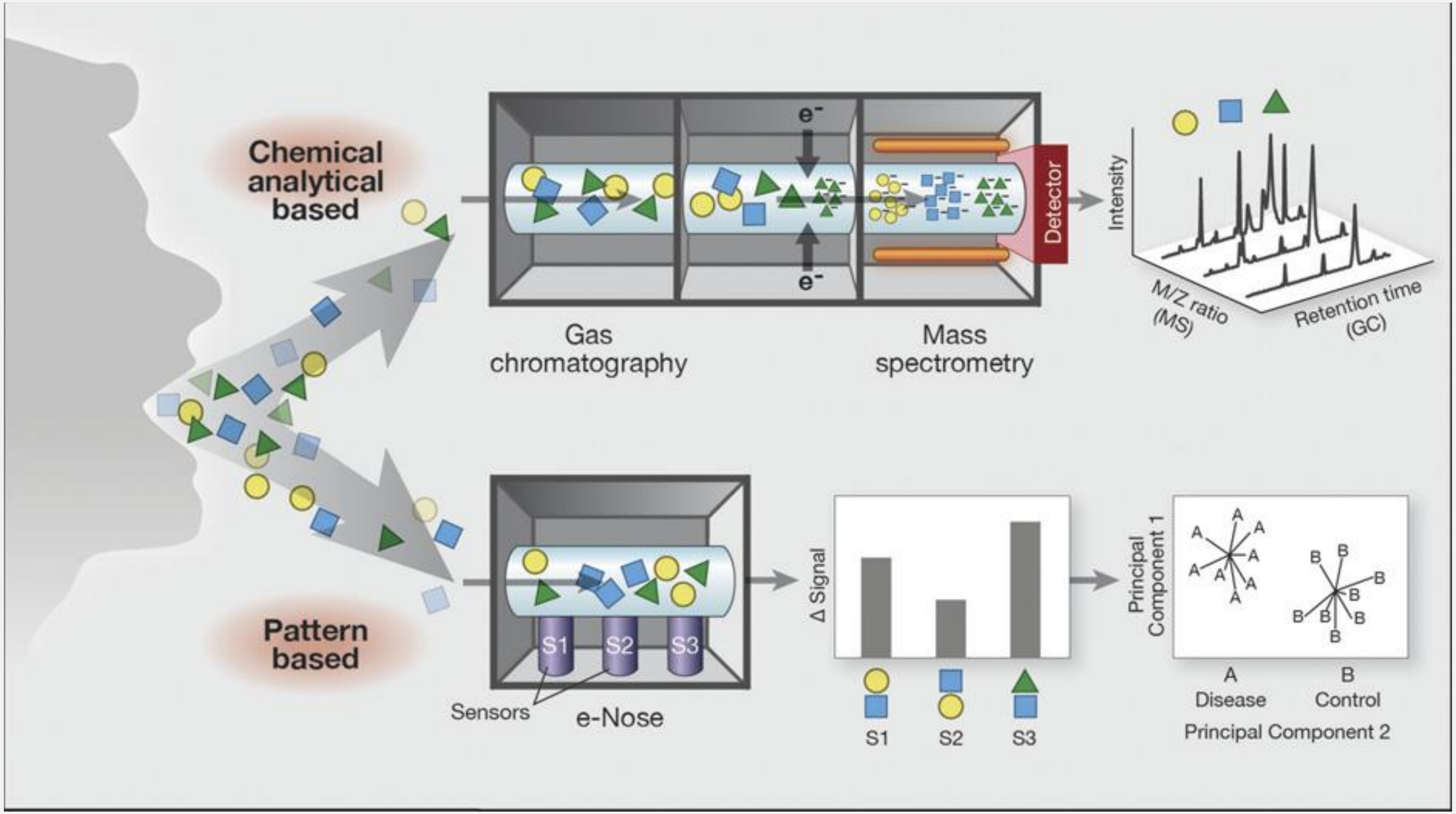
ACS Nano 2017;11:112





Average accuracy: 86%

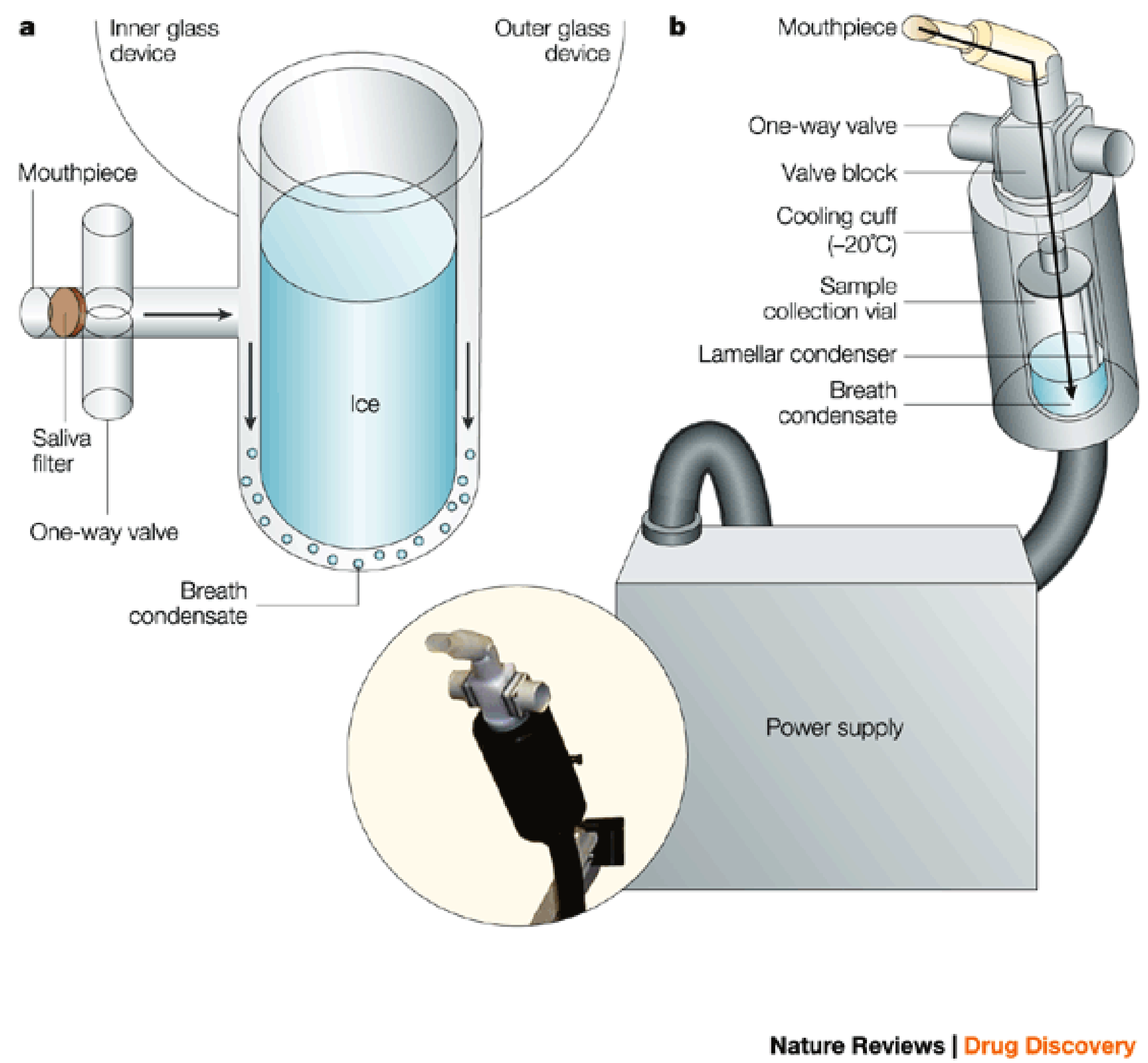
ACS Nano 2017;11:112



Chest 2015;247:224

	GC	E-Nose
Pro	Detection of specific VOCs No device training necessary Standardized technique Required for pathophysiological research	Cheap Portable Fast Possibility of direct measurement Able to detect VOCs in low concentration Suitable for clinical application based on probabilistic evidence
Con	Expensive Requires a lab and skilled personnel Takes more time Trapping on sorbent tube necessary Difficulties detecting VOCs in low concentrations	No detection of disease-specific VOCs Device training necessary Standardization needed Breathprints between different eNose types not comparable

EBC

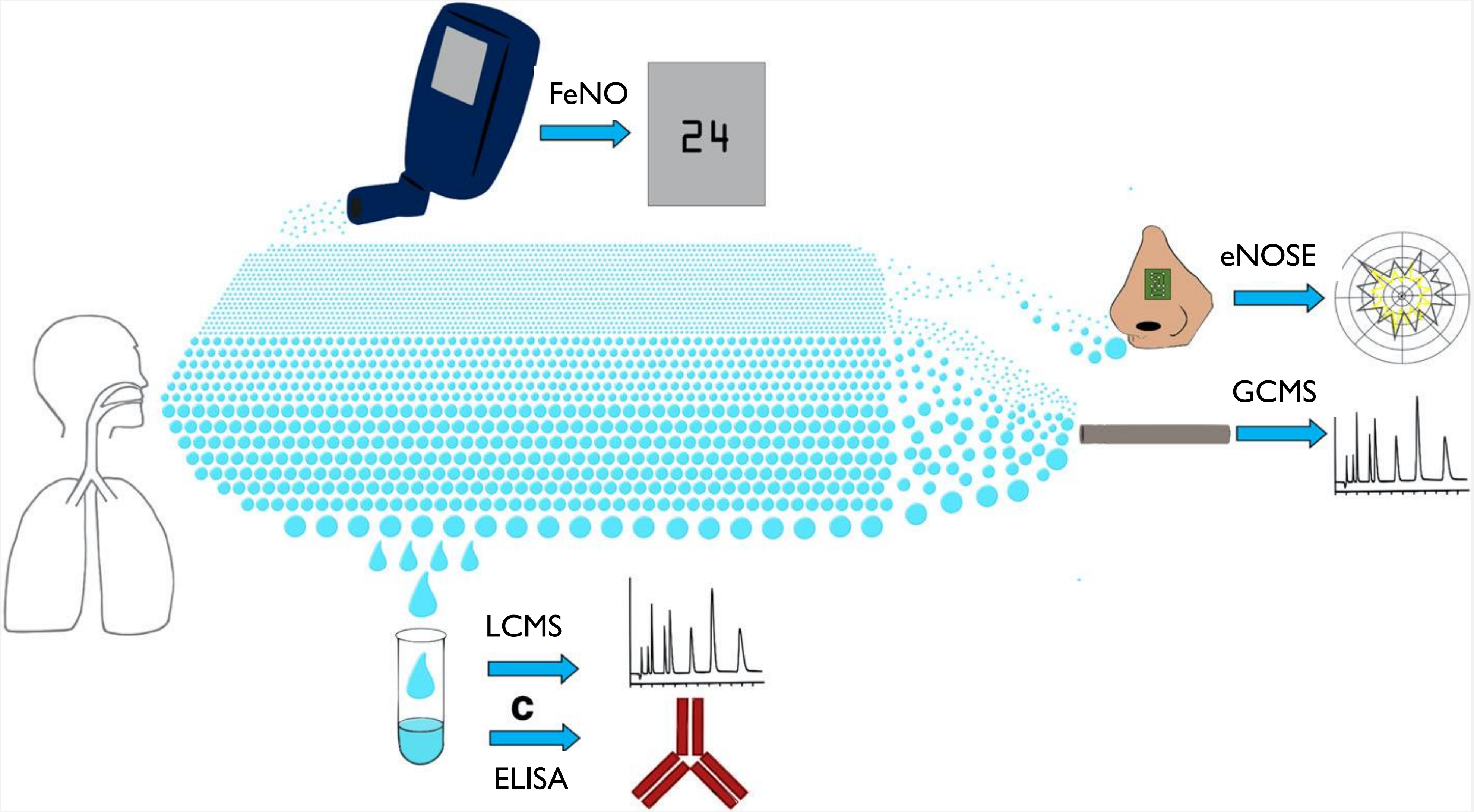

a
Inner glass device
Outer glass device
Mouthpiece
Saliva filter
One-way valve
Ice
Breath condensate
b
Mouthpiece
One-way valve
Valve block
Cooling cuff (–20°C)
Sample collection vial
Lamellar condenser
Breath condensate
Power supply
Nature Reviews | Drug Discovery


R
TUBE

EBC MEASUREMENTS

- pH
- H2O2
- LTs
- 8-isoprostane
- PGE2

- ILs
- TNFa
- TGFb
- RANTES
- CRP



Respiratory Res 2019:20

FDA APPROVED BREATH TESTS

- Breath hydrogen test for carbohydrate metabolism
- Breath nitric oxide test to monitor therapy for asthma
- Breath carbon monoxide test for neonate jaundice
- Breath test for diagnosis of *H. pylori*
- Breath test for heart transplant rejection
- Breath ethanol for intoxication (law enforcement)

Exhaled Breath Profiling Enables Discrimination of Chronic Obstructive Pulmonary Disease and Asthma

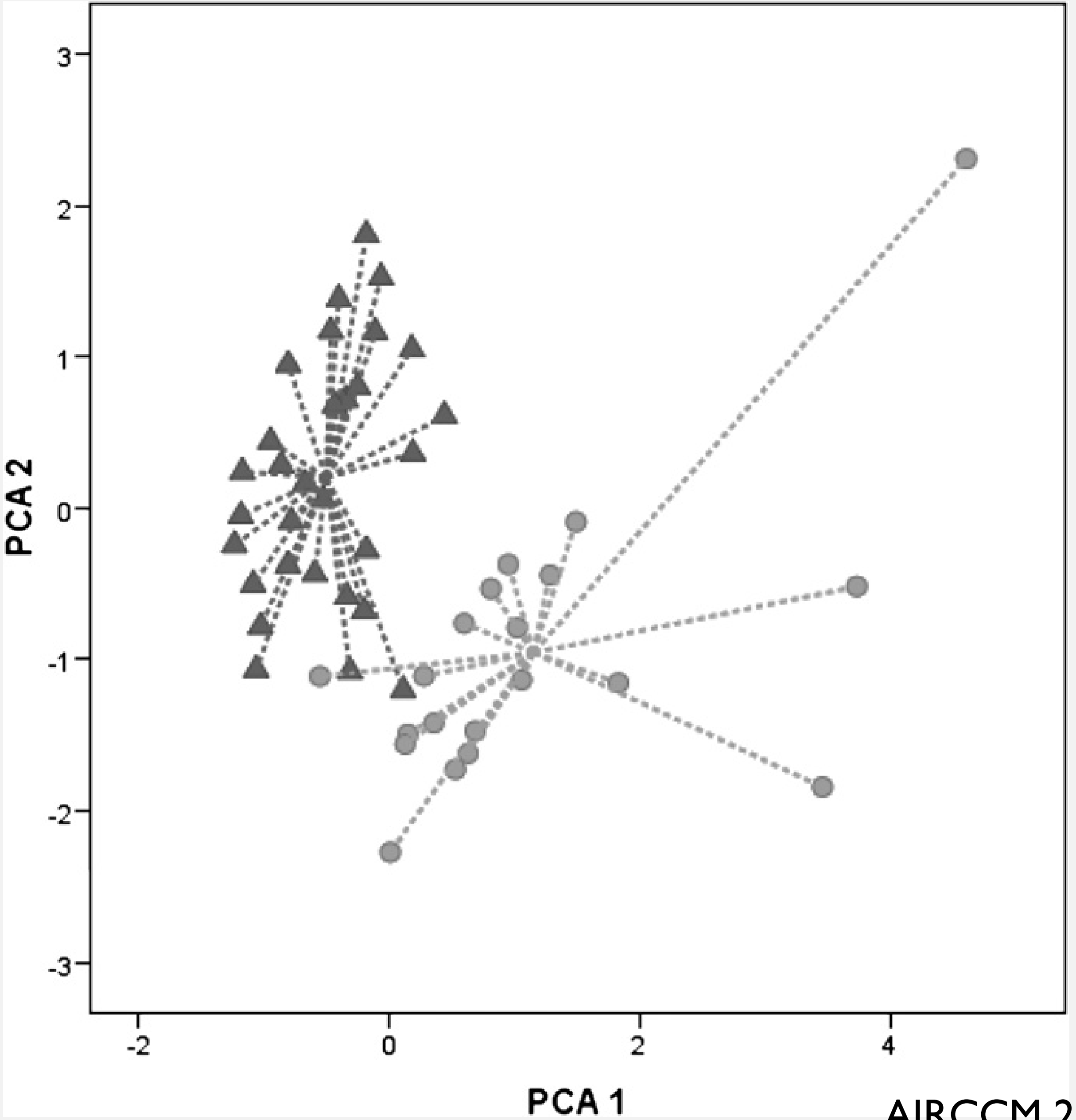
Niki Fens[1], Aeilko H. Zwinderman[2], Marc P. van der Schee[1], Selma B. de Nijs[1], Erica Dijkers[1], Albert C. Roldaan[3], David Cheung[4], Elisabeth H. Bel[1], and Peter J. Sterk[1]

[1]Department of Respiratory Medicine and [2]Clinical Epidemiology and Biostatistics, Academic Medical Centre, University of Amsterdam, Amsterdam; [3]Department of Pulmonary Diseases, Haga Teaching Hospital Leyweg, Den Haag; and [4]Department of Respiratory Medicine, Albert Schweitzer Hospital, Dordrecht, The Netherlands

AJRCCM 2009;180:1076

Subject Characteristics	COPD	Asthma	Nonsmoking Control Subjects	Smoking Control Subjects
n	30	20	20	20
Age, years	61.6 ± 9.3*	35.4 ± 15.1	56.7 ± 9.3	56.1 ± 5.9
Sex (M/F)	17/13	8/12	10/10	10/10
FEV_1 postbronchodilator, L	1.72 ± 0.69	3.32 ± 0.86	3.44 ± 0.76	3.58 ± 0.78
FEV_1 postbronchodilator, % pred	57 ± 15	95 ± 18	116 ± 15	112 ± 14
FEV_1/FVC	0.45 ± 0.12	0.76 ± 0.11	0.78 ± 0.07	0.77 ± 0.05
GOLD-stage (II/III)	20/10	NA	NA	NA
GINA-classification Mild/moderate/severe, n	NA	11/5/4	NA	NA
Pack-years	42.8 ± 17.7	0.6 ± 1.2	0.1 ± 0.2	37.9 ± 17.1
Current/ex-/never-smoker, (n)	9/21/0	0/4/16	0/1/19	20/0/0
ICS use, n	13	18	0	0

AJRCCM 2009;180:1076

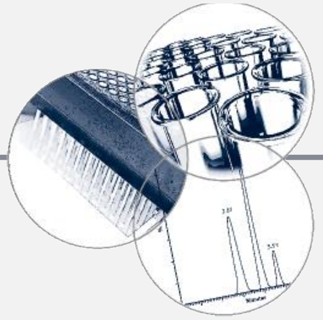


AJRCCM 2009;180:1076

Analysis	Cross-validated Accuracy (%)	*P* Value
Asthma–COPD	96	<0.0001
Asthma–COPD smoking	97	<0.0001
Asthma–COPD ex-smoking	95	<0.0001
Asthma–COPD ICS	97	<0.0001
Asthma–COPD no ICS	95	<0.0001
Asthma–nonsmoking control subjects	95	<0.0001
COPD–smoking control subjects	66	0.006
COPD smoking–smoking control subjects	72	0.018
COPD ex-smoking–smoking control subjects	61	0.026
COPD ICS–smoking control subjects	70	0.024
COPD no ICS–smoking control subjects	65	0.047
Control subjects–smoking control subjects	63	0.016

AJRCCM 2009;180:1076

Research Article



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Metabolomics pilot study to identify volatile organic compound markers of childhood asthma in exhaled breath

Background: In-community non-invasive identification of asthma-specific volatile organic compounds (VOCs) in exhaled breath presents opportunities to characterize phenotypes, and monitor disease state and therapies. The feasibility of breath sampling with children and the preliminary identification of childhood asthma markers were studied. **Method:** End-tidal exhaled breath was sampled (2.5 dm^3) from 11 children with asthma and 12 healthy children with an adaptive breath sampler. VOCs were collected onto a Tenax®/Carbotrap hydrophobic adsorbent trap, and analyzed by GC–MS. Classification was by retention-index and mass spectra in a 'breath matrix' followed by multivariate analysis. **Results:** A panel of eight candidate markers (1-(methylsulfanyl)propane, ethylbenzene, 1,4-dichlorobenzene, 4-isopropenyl-1-methylcyclohexene, 2-octenal, octadecyne, 1-isopropyl-3-methylbenzene and 1,7-dimethylnaphtalene) were found to differentiate between the asthmatic and healthy children in the test cohort with complete separation by 2D principal components analysis (2D PCA). Furthermore, the breath sampling protocol was found to be acceptable to children and young people. **Conclusion:** This method was found to be acceptable for children, and healthy and asthmatic individuals were distinguished on the basis of eight VOCs at elevated levels in the breath of asthmatic children.

Bioanalysis 2013;5:2239

Table 1. Characteristics of study participants.

Variable	Asthma (n = 11)	Controls (n = 12)	p-value
Age (years)†	12 ± 1.8	12 ± 2.7	1.000
Height (cm)†	156 ± 12.4	155 ± 16.4	0.878
Weight (kg)†	50.8 ± 15.6	50.7 ± 17.2	0.995
BMI (kg/m^2)†	20.5 ± 3.8	20.4 ± 3.5	0.960
Male/female (n)	8/3	8/4	NA
FEV1, Z-score†	-0.83 ± 1.01	-0.03 ± 0.70	0.0219
FeNO (ppb)‡	52 (12–219)	14 (8–70)	0.0210
Atopy (SPT+)	8 (73%)	0	NA
ICS	9 (82%)	0	NA
2000 µg§	2	NA	NA
1000 µg§	4	NA	NA
400–800 µg§	3	NA	NA
Montelukast	8 (72%)	NA	NA
LABA	9 (92%)	NA	NA

Bioanalysis 2013;5:2239

Table 2. Volatile organic compounds that differentiate between healthy and asthmatic children.

Assignment	RIB	RIs	MatchF	MatchR	P (%)
1-(methylsulfanyl)propane	851	NA	NA	NA	NA
Ethylbenzene†	904	860	786	854	98.0
1,4-dichlorobenzene	1014	1020	825	835	98.9
4-isopropenyl-1-methylcyclohexene†	1029	1030	828	850	98.8
2-octenal†	1032	1059	749	749	98.8
octadecyne	1104	NA	NA	NA	NA
1-isopropyl-3-methylbenzene	1114	1026	712	753	92.3
1,7-dimethylnaphtalene	1424	1418	672	703	87.5

Bioanalysis 2013;5:2239

Journal of Breath Research

TOPICAL REVIEW

A systematic review of breath analysis and detection of volatile organic compounds in COPD

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Keywords: chronic obstructive pulmonary disease, biomarker, volatile organic compound, bioinformatics, machine learning, breath analysis

Supplementary material for this article is available online

J Breath Res 2016:10;034002

VOCS RELATED WITH COPD

- ORGANIC ACIDS
  - ACETIC ACID, BUTANOIC ACID, PENTANOIC ACID
- ALCOHOLS
  - 2-PROPANOL, CYCLOHEXANOL, PHENOL, TERPINEOL
- ALDEHYDES
  - BENZALDEHYDE, DODECANAL, HEXANAL, NONANAL, PENTADECANAL, UNDECANAL
- HYDROCARBONS
  - BUTYLBENZENE, PENTENE, DECANE, HEPTANE, OCTATRIENE, BENZENE, BUTANE, DECANE, HEXADECANE, LIMONENE, NONADECANE, OCTADECANE, TOLUENE, UNDECANE
- OTHERS
  - PENTYLFURAN, BENZOFURAN, BENZONITRILE, INDOLE, SULPHUR DIOXIDE

J Breath Res 2016:10;034002

Journal of
Clinical Medicine

MDPI

Article

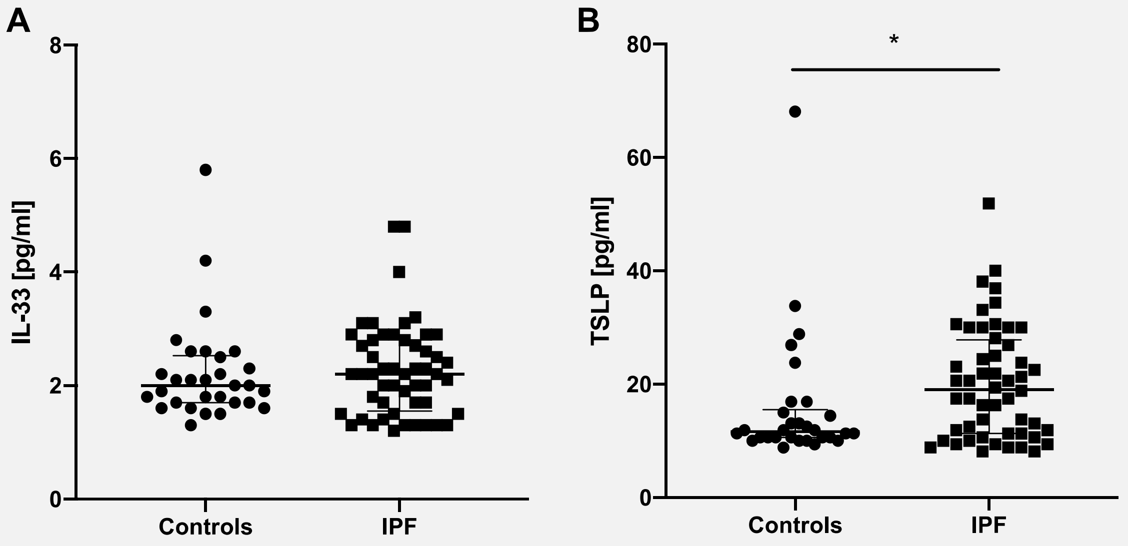
Epithelial Alarmins in Serum and Exhaled Breath in Patients with Idiopathic Pulmonary Fibrosis: A Prospective One-Year Follow-Up Cohort Study

Table 1. Characteristics of study participants.

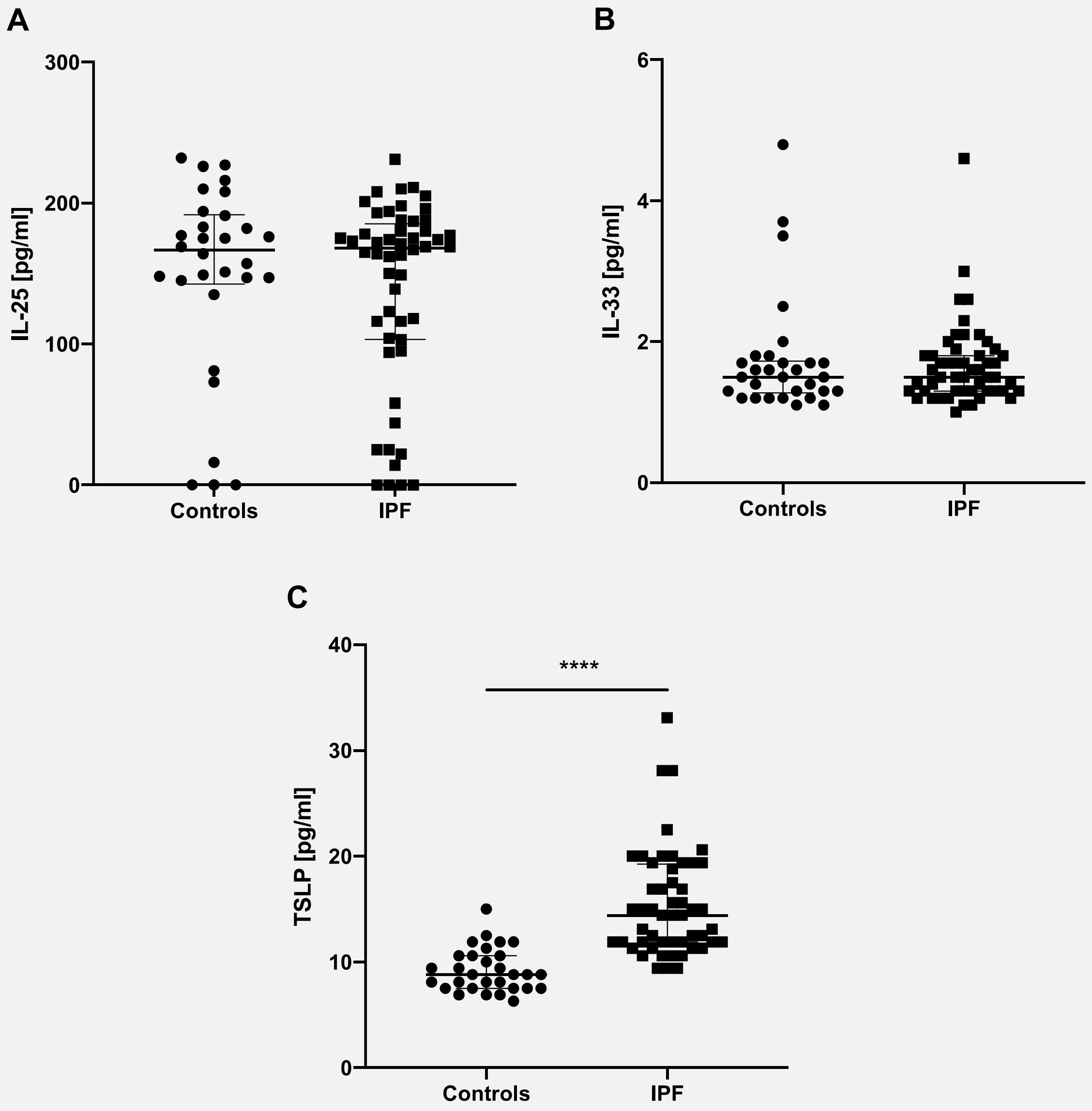
	Controls	IPF
Number of subjects	30	52
Sex (male/female)	17/13	39/13
Age (years), mean (SD)	61.36 (7.26)	68.21 (8.76) ***
Smoking exposure (pack-years) median (IQR)	8 (0–25)	26.5 (7.75–39) **
Smoking status		
never smokers, *n* (%)	12 (40.00)	12 (23.07)
ex-smokers, *n* (%)	8 (26.67)	37 (71.15)
current smokers, *n* (%)	10 (33.33)	3 (5.77)
GAP score, median (IQR)	N/A	4.0 (3.0–4.5)

J CLIN MED 2019

SERUM

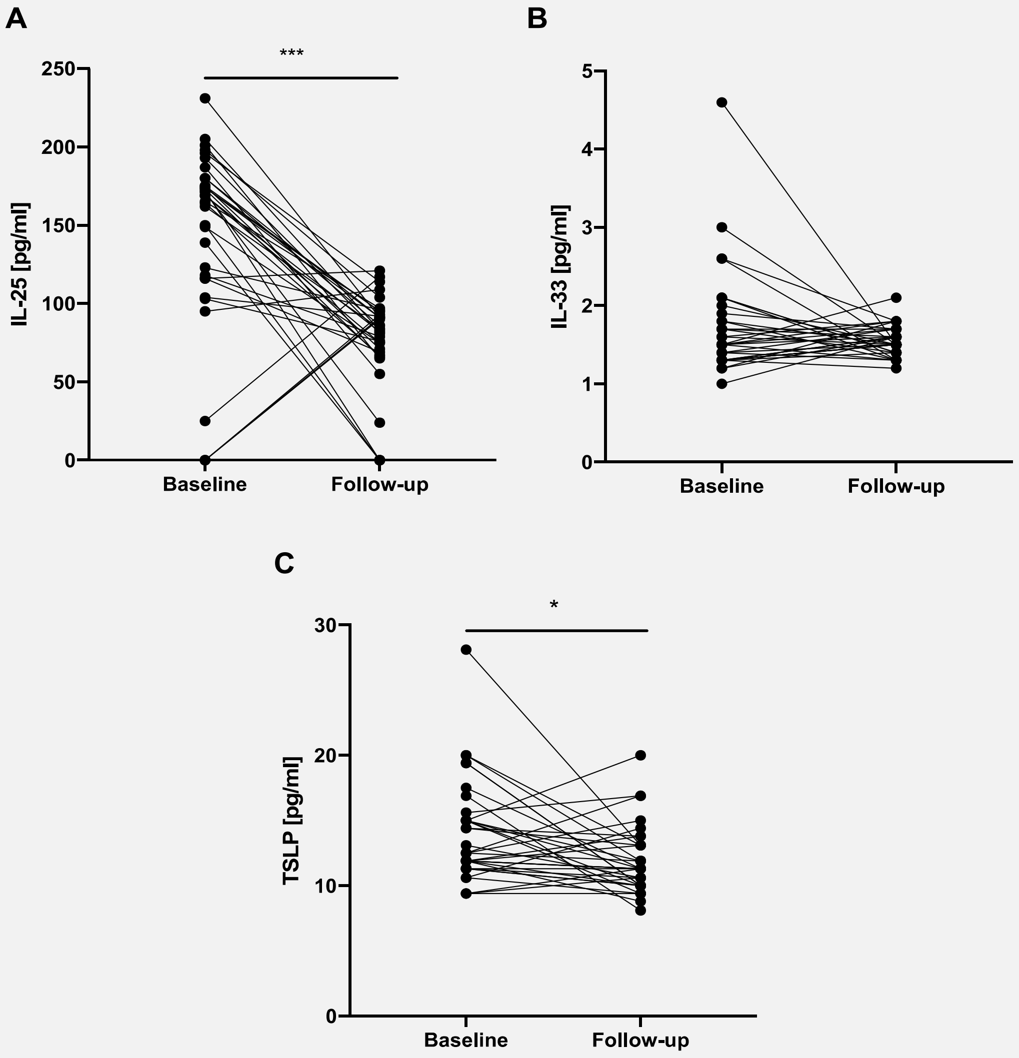


EBC



J CLIN MED 2019

AFTER 12 MONTHS OF ANTI-FIBROTIC THERAPY



J CLIN MED 2019

REVIEW

Open Access

Breath biomarkers in idiopathic pulmonary fibrosis: a systematic review

Conal Hayton[1,3*], Dayle Terrington[2], Andrew M. Wilson[2], Nazia Chaudhuri[3], Colm Leonard[3] and Stephen J. Fowler[1,3]

Abstract

Background: Exhaled biomarkers may be related to disease processes in idiopathic pulmonary fibrosis (IPF) however their clinical role remains unclear. We performed a systematic review to investigate whether breath biomarkers discriminate between patients with IPF and healthy controls. We also assessed correlation with lung function, ability to distinguish diagnostic subgroups and change in response to treatment.

Methods: MEDLINE, EMBASE and Web of Science databases were searched. Study selection was limited to adults with a diagnosis of IPF as per international guidelines.

Results: Of 1014 studies screened, fourteen fulfilled selection criteria and included 257 IPF patients. Twenty individual biomarkers discriminated between IPF and controls and four showed correlation with lung function. Meta-analysis of three studies indicated mean (± SD) alveolar nitric oxide ($C_{alv}NO$) levels were significantly higher in IPF (8.5 ± 5.5 ppb) than controls (4.4 ± 2.2 ppb). Markers of oxidative stress in exhaled breath condensate, such as hydrogen peroxide and 8-isoprostane, were also discriminatory. Two breathomic studies have isolated discriminative compounds using mass spectrometry. There was a lack of studies assessing relevant treatment and none assessed differences in diagnostic subgroups.

Conclusions: Evidence suggests $C_{alv}NO$ is higher in IPF, although studies were limited by small sample size. Further breathomic work may identify biomarkers with diagnostic and prognostic potential.

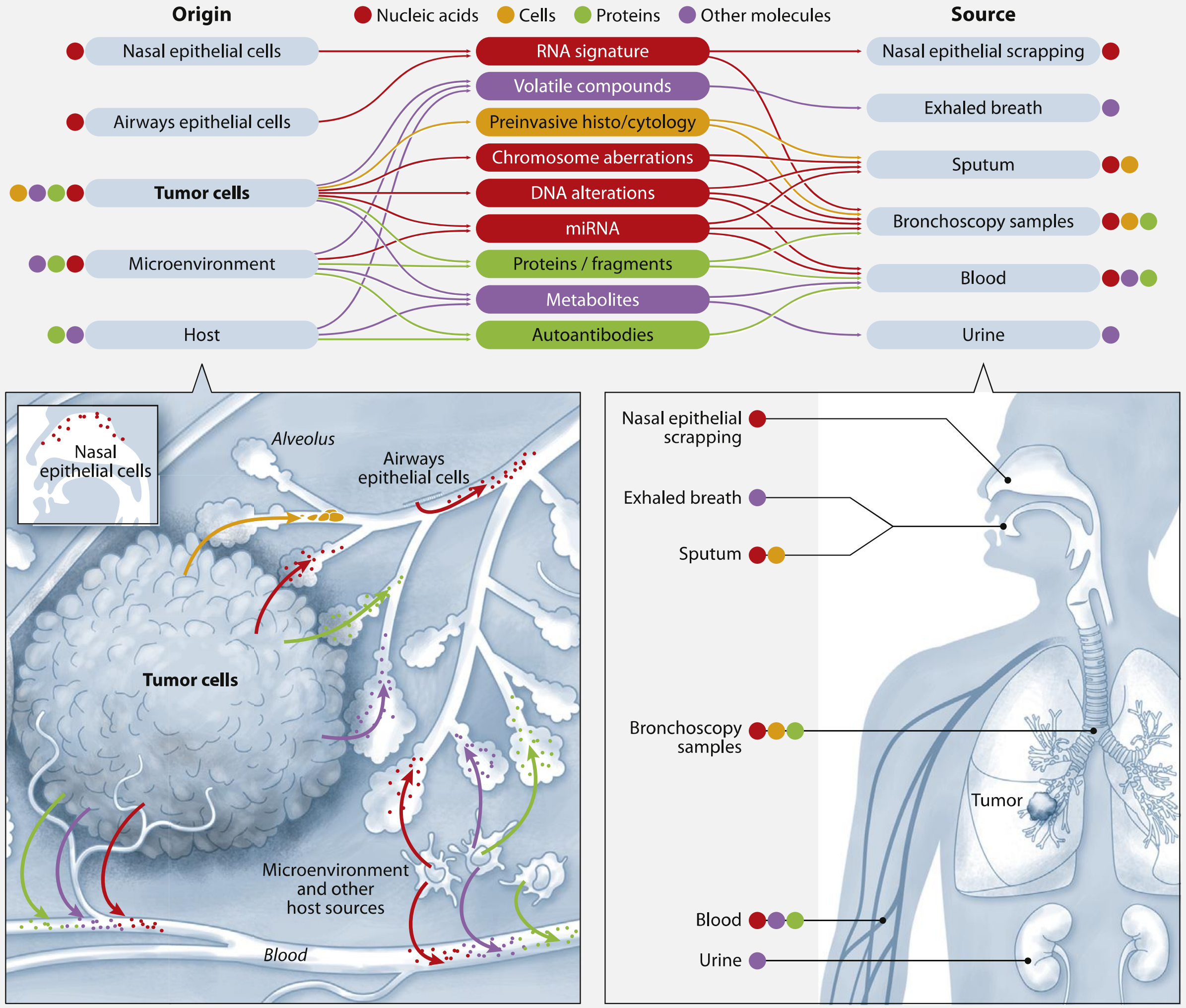
Keywords: Idiopathic pulmonary fibrosis, Breath tests, Nitric oxide, Volatile organic compounds, Exhaled breath condensate

Table 2 Biomarkers reported to discriminate between IPF patients and healthy controls. Direction of discrimination and reported *p*-value. [a]$C_{alv}NO$. [b]$FeNO_{50}/FeNO_{100}/FeNO_{150}/C_{alv}NO$

Biomarker	Sample Medium	Discrimination	*p*-value	References
Nitric Oxide	Exhaled breath	Higher in IPF	0.0001, < 0.0001	[62][a], [65][b]
8-isoprostane	EBC	Higher in IPF	0.02, < 0.05	[58], [62]
Hydrogen Peroxide	EBC	Higher in IPF	0.003	[58]
Nickel	EBC	Higher in IPF	< 0.05	[59]
Chromium	EBC	Higher in IPF	< 0.05	
Silicon	EBC	Higher in IPF	< 0.05	
Cobalt	EBC	Lower in IPF	< 0.05	
Iron	EBC	Lower in IPF	< 0.05	
Copper	EBC	Lower in IPF	< 0.05	
Selenium	EBC	Lower in IPF	< 0.05	
Molybdenum	EBC	Lower in IPF	< 0.05	
Nitrite	EBC	Higher in IPF	< 0.01	[60]
Nitrate	EBC	Lower in IPF	< 0.01	
22:4 LPA	EBC	Higher in IPF	0.001	[63]
Unidentifiable metabolite	EBC	Higher in IPF	≤0.01	[64]
p-cymene	Exhaled breath	Lower in IPF	< 0.001	[66]
Acetoin	Exhaled breath	Higher in IPF	< 0.001	
Isoprene	Exhaled breath	Higher in IPF	< 0.001	
Ethylbenzene	Exhaled breath	Higher in IPF	< 0.001	
Unidentified VOC	Exhaled breath	Higher in IPF	< 0.001	

IPF idiopathic pulmonary fibrosis, *EBC* exhaled breath condensate, *22:4 LPA* Docosatetraenoyl lypophosphatidic acid, *VOC* volatile organic compound, $C_{alv}NO$ alveolar nitric oxide concentration, $FeNO_{50/100/150}$ fractionated exhaled nitric oxide at 50 ml/100 ml/150 ml per second

BIOMARKERS OF LUNG CANCER



Journal of Thoracic Oncology 2018:14;343

> J Breath Res. 2020 Jul 24;14(4):046004. doi: 10.1088/1752-7163/ab8c50.

Recognition of breathprints of lung cancer and chronic obstructive pulmonary disease using the Aeonose ® electronic nose

Ekaterina Krauss [1], Jana Haberer, Guillermo Barreto, Maria Degen, Werner Seeger, Andreas Guenther

Affiliations + expand

PMID: 32325432 DOI: 10.1088/1752-7163/ab8c50

- 120 lung cancer patients
- 33 healthy controls
- 23 COPD patients

  - Separation of lung cancer and healthy controls
    - AUC 0.92
    - Sensitivity 0.84
    - Specificity 0.97
  - Recognized 11/24 COPD patients as being lung cancer (+)

J Breath Res. 2020;14:046004

SUMMARY

- There are many potential breath biomarkers that are useful in diagnosing and assessing respiratory diseases.
- Spectrometric analyses/sensor-based analyses (eNose)/EBC
- Standardization of collection, processing and analysis is required