Breathomics: potential applications in respiratory and occupational medicine

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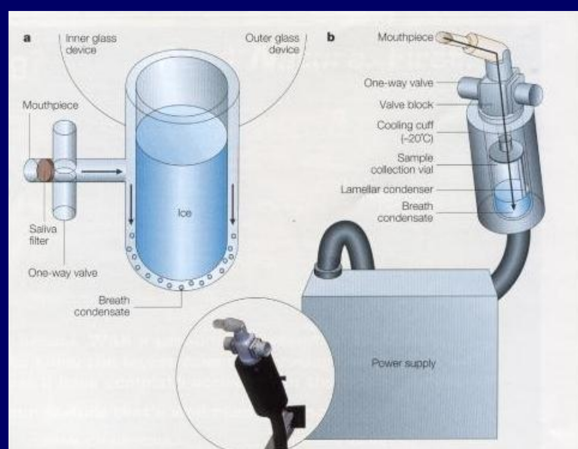
Non-invasive monitoring of lung inflammation

F_ENO



EBC

induced sputum

e-nose

F_ENO analysers

NIOX®

NIOX MINO®

Both devices cleared by the US FDA for monitoring therapy in atshma

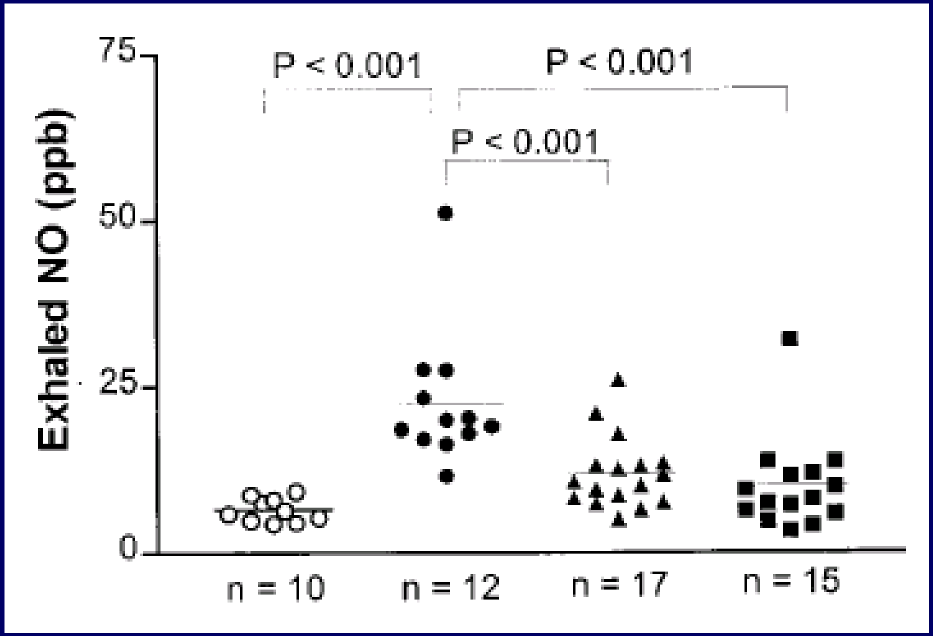
F_ENO: advantages

- Standardized
- Noninvasive
- Reproducible
- Rapid test with immediate result
- Approved in the clinical setting
- Correlate with eosinophilic inflammation and AHR before steroid treatment
- Useful in diagnosis of asthma
- Decreses rapidly after anti-inflammatory medications and predicts response
- Increases during asthma exacerbations
- Can be used to monitor compliance

modified from Bates C.A. and Silkoff P.E., JACI, 2003

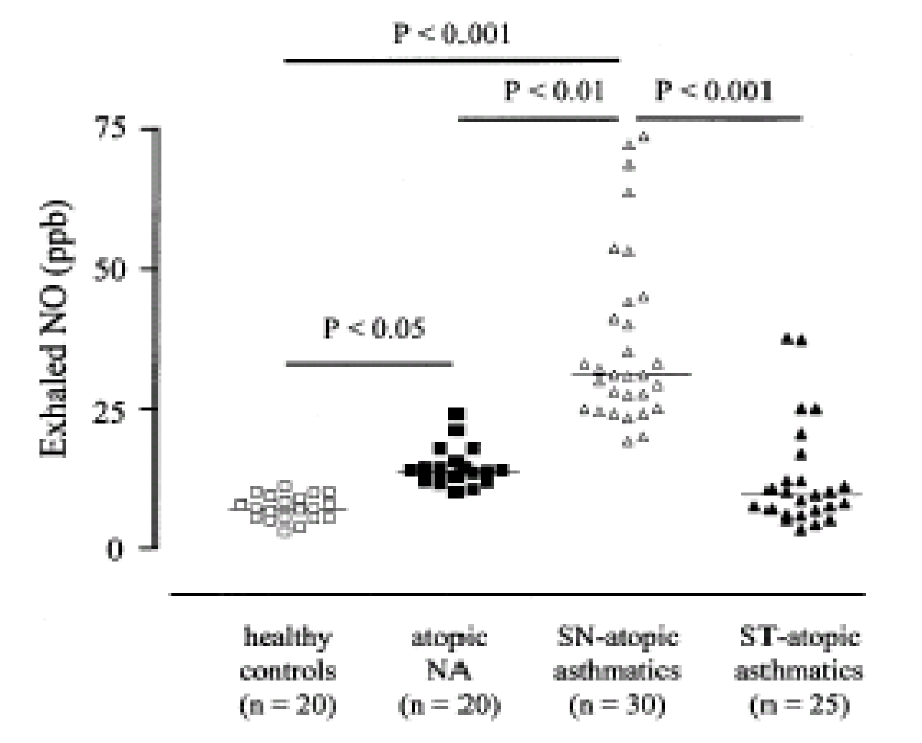
F_ENO in asthma

Adults



Montuschi P. et al, AJRCCM, 1999

Children

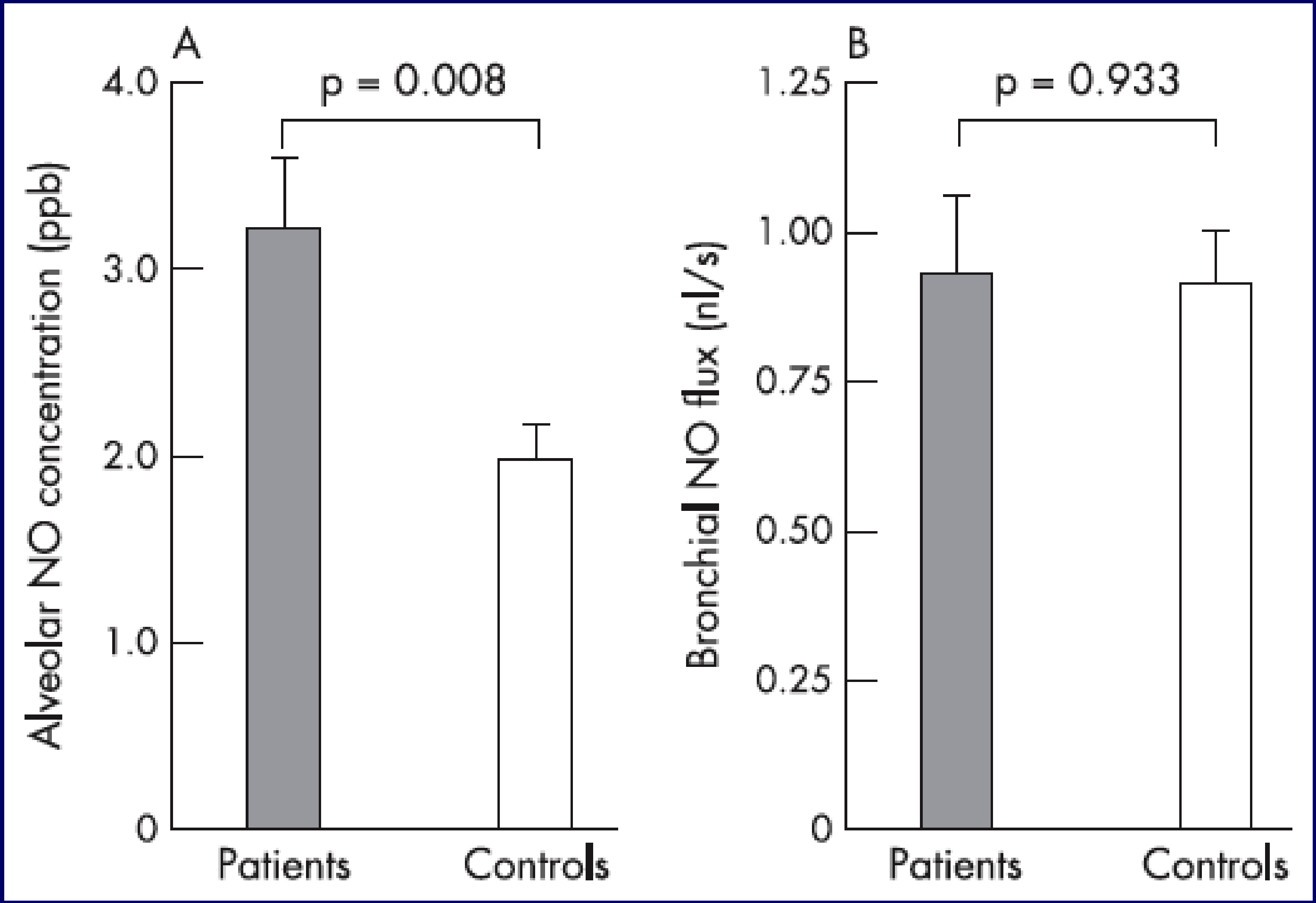


Mondino C. et al, JACI, 2004

Exhaled nitric oxide in occupational medicine

- occupational exposure
  - sensitizers (occupational asthma)
    - latex, isocyanates
  - irritants
    - asbestos
    - toluene, xylene, methylketone
    - dust, gases (concrete workers)
- general population exposure
  - irritants
    - ozone (short-term exposure)
    - thrichloramine (chronic exposure)

F_ENO in workers previously exposed to asbestos



15 15 15 15

average exposure 20.5 years

exposure stopped 25.6 years before

Lehtonen H. et al, Thorax, 2007

$F_E NO$:
an example of translational medicine

From basic research

Gustaffson LE et al. Endogenous nitric oxide is present in the exhaled air of rabbits, guinea pigs and humans. *Biochem Biophys Res Commun* 1991;181: 852-857.

Through validation of the technique

Recommendations for standardized procedures for the on-line and off-line measurement of exhaled lower respiratory nitric oxide and nasal nitric oxide in adults and children-1999. This official statement of the American Thoracic Society was adopted by the ATS Board of Directors, July 1999. *Am J Respir Crit Care Med* 1999;160:2104-2117.

To clinical setting

Silkoff PE et al. The Aerocrine exhaled nitric oxide monitoring system NIOX is cleared by the US Food and Drug Administration for monitoring therapy in asthma. *J Allergy Clin Immunol* 2004;114:1241-1256.

Breath condensate analysis

Breath

- gaseous phase

  (NO, CO)

- liquid phase

  water vapor

  aerosol particles

  (inflammatory mediators)

Advantages

- easy to perform

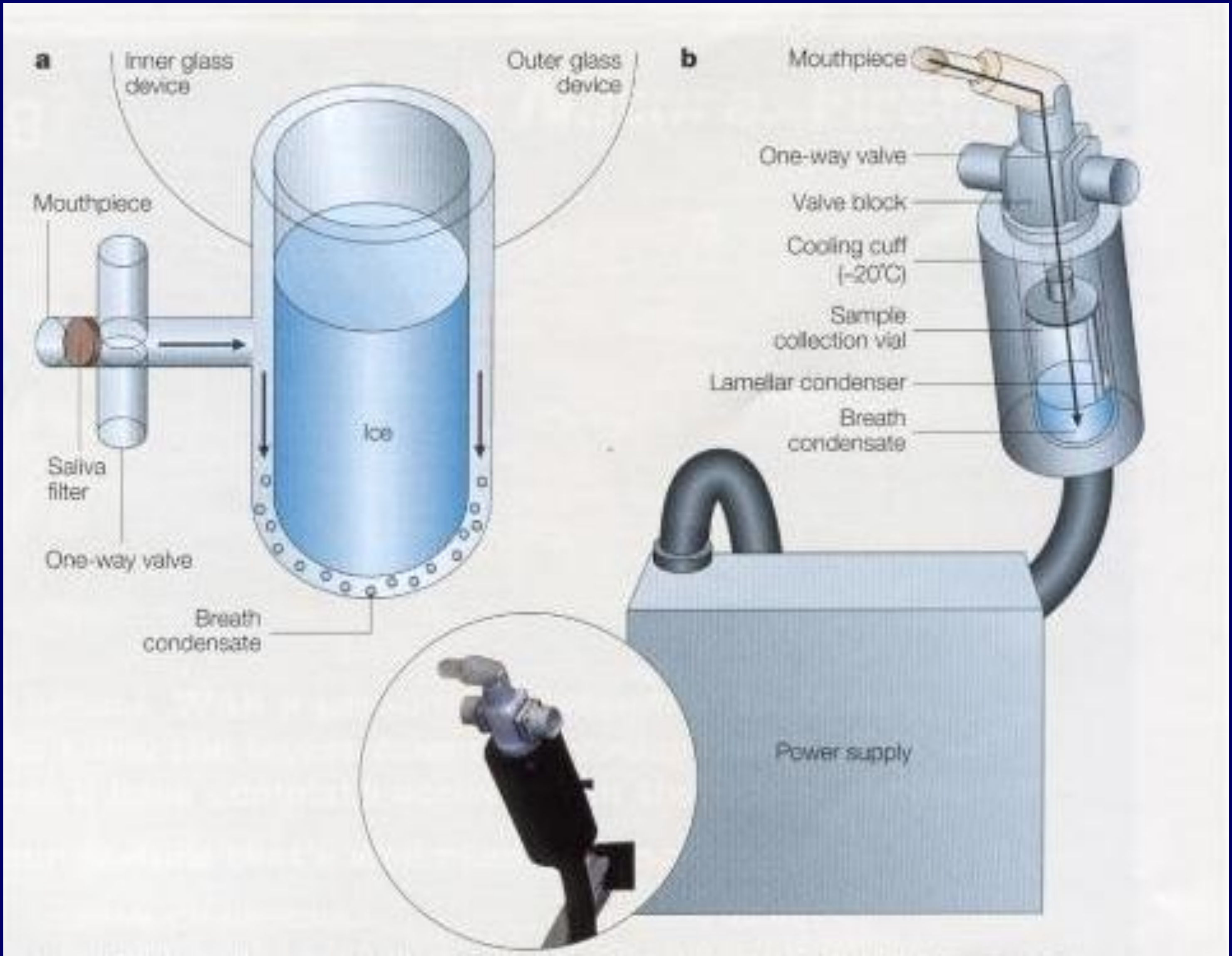
  (severe airway inflammatory diseases, children)

- repeated measures
- reflects lung inflammation

Disadavantages

- no cell analysis

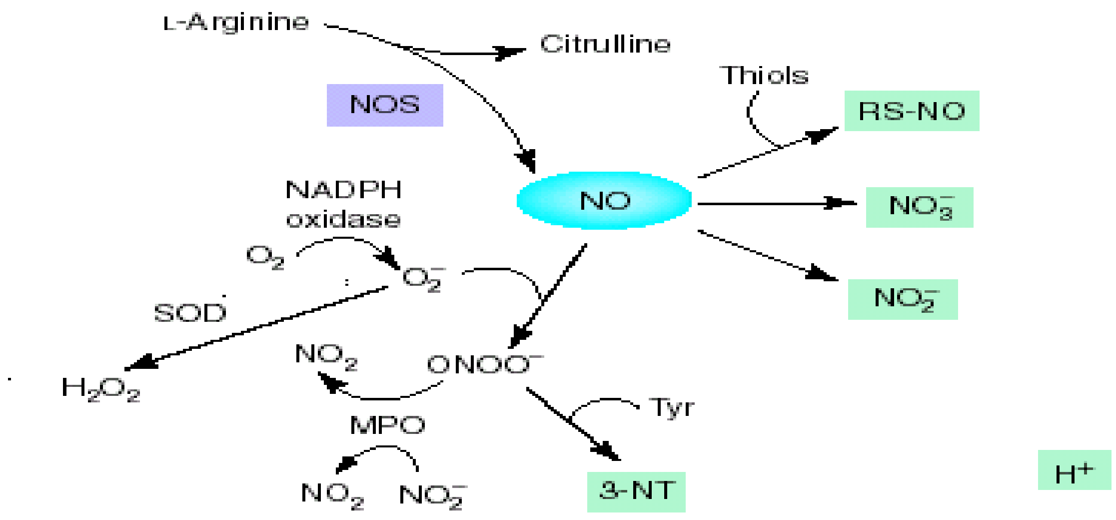
Breath condensate analysis: equipments



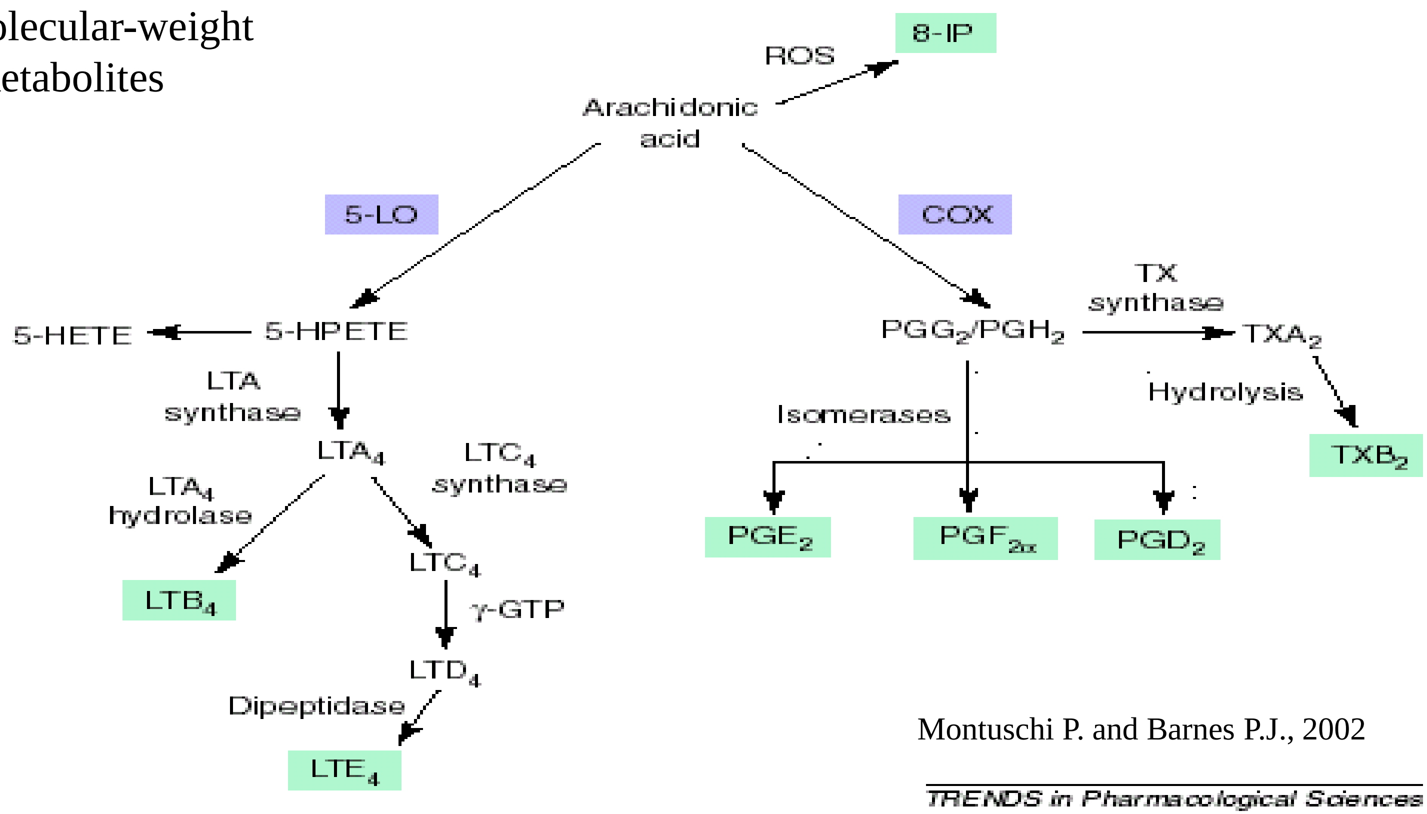
Montuschi P., Nature Reviews Drug Discovery, 2002

Breath condensate analysis: equipments

(by courtesy of Dr. John Hunt and John Vaughan) adenosine



low molecular-weight metabolites



Montuschi P. and Barnes P.J., 2002

Mass spectrometry of eicosanoids in EBC: LTB_4, 8-isoprostane, LTD_4, LTE_4, PGE_2

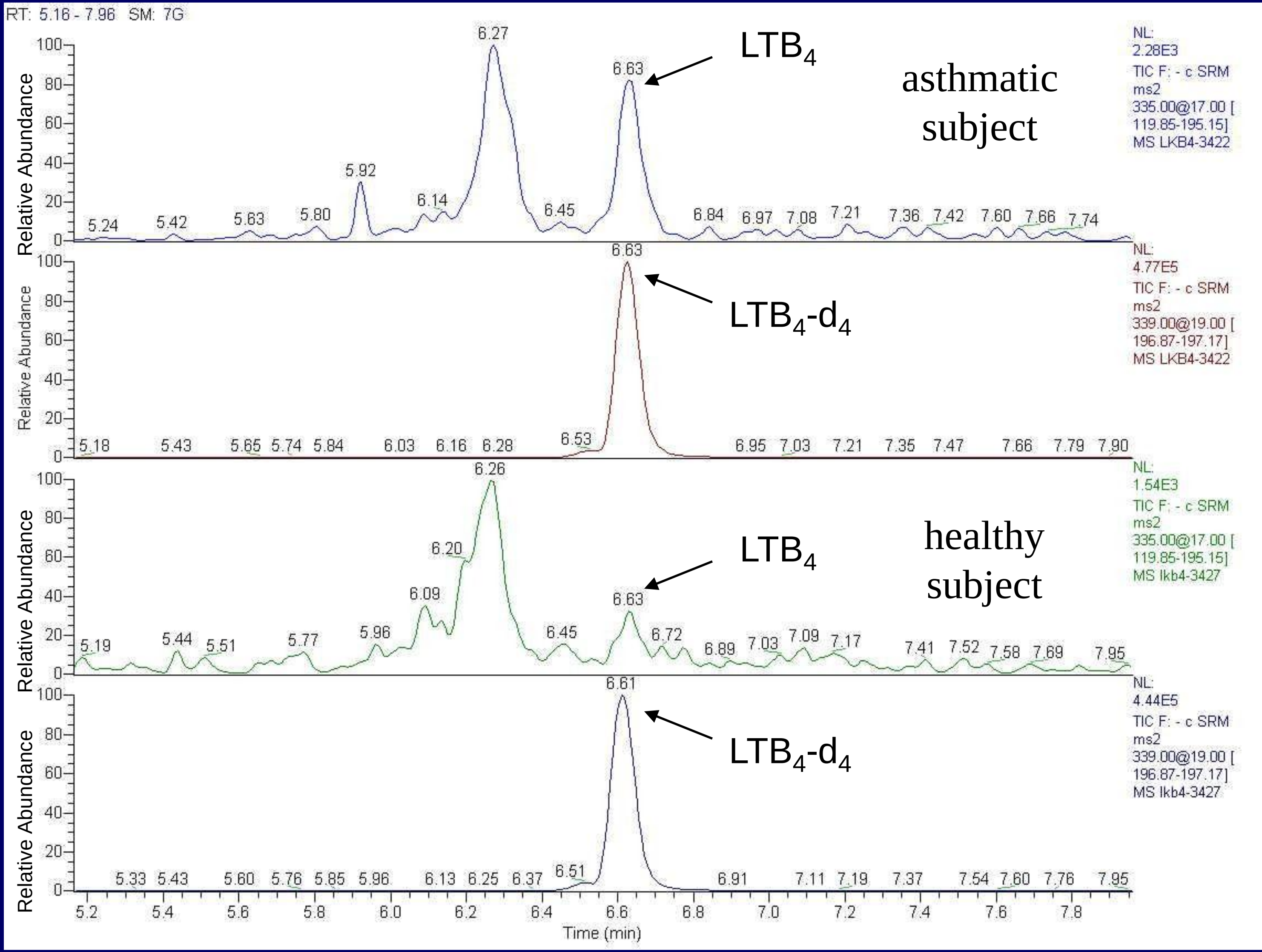
LC/MS techniques

- Montuschi P et al., Rapid Commun Mass Spectrom 2004;18:2723-2729
- Montuschi P et al., Respir Res 2005;6:119
- Montuschi P et al, J Chromatogr B Analyt Technol Biomed Life Sci. 2009;877:1272-80
- Syslova K et al, J Chromatogr B Analyt Technol Biomed Life Sci 2009;877:2477-2486
- Syslova K et al, J Chromatogr B Analyt Technol Biomed Life Sci 2008;867:8-14
- Gonzalez-Reche LM et al, J Occup Med Toxicol 2006;1:5

GC/MS techniques

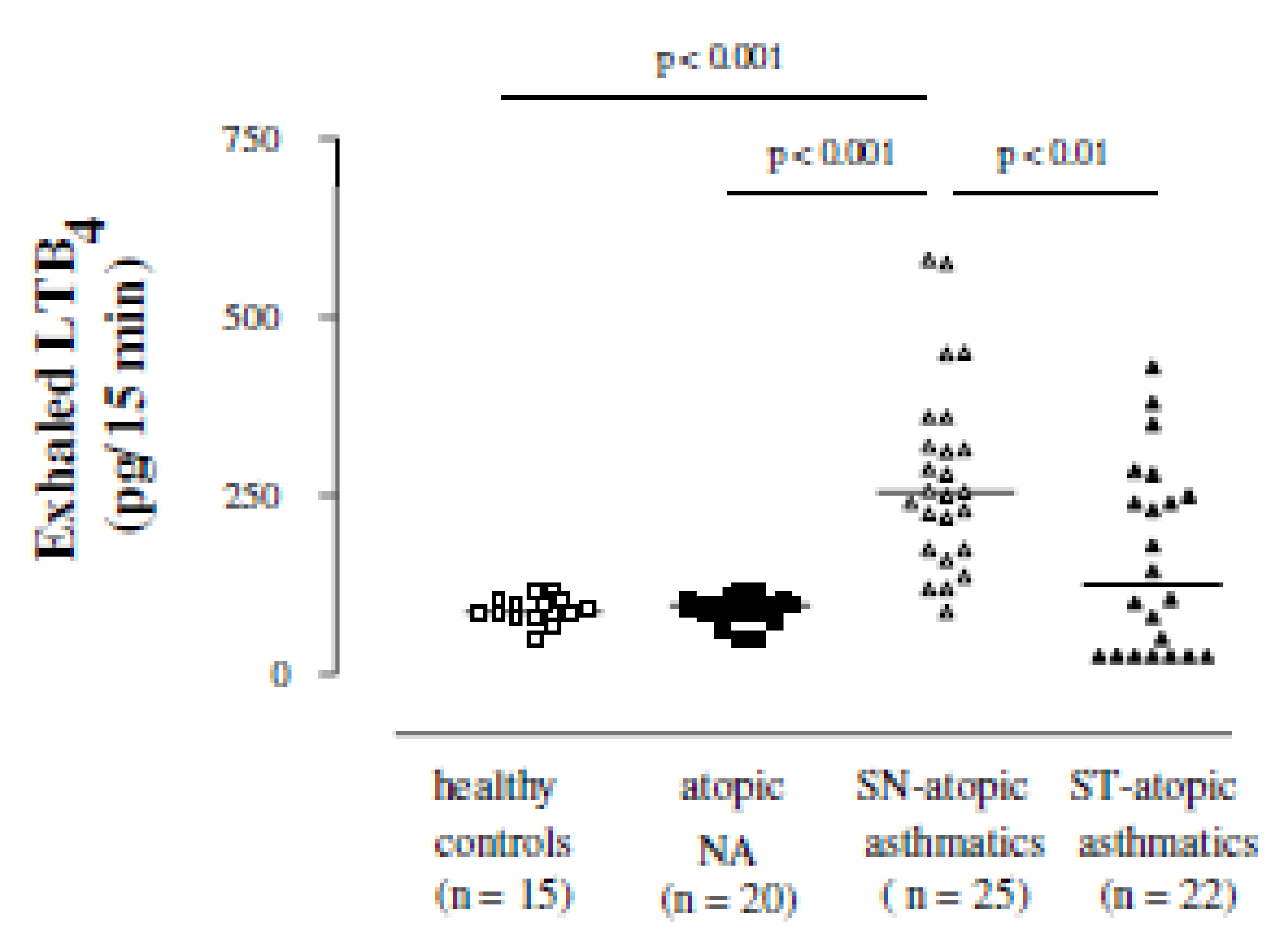
- Cap P et al, Thorax 2004;59:465-470
- Carpenter CT et al, Chest 1998;14:1653-1659
- Kielbasa B et al, Pediatr Allergy Immunol 2008;19:660-669
- Sanak M et al, Clin Exp Allergy 2004;34:1899-904

LTB_4 in EBC in asthma: LC/MS/MS



Montuschi P. et al, Respir. Res., 2005

LC/MS/MS of LTB_4 in EBC in asthmatic children



Montuschi P. et al., Respir. Res., 2005

EBC in occupational medicine

- 8-IsoP, LTB_4, PGE_2, pH in EBC to monitor the effects of welding fume exposure (depending on welding techniques) (Hoffmeyer F et al, J Toxicol Environ Health A 2012;75:525-32)
- 8-IsoP, LTB_4, PGE_2, pH in EBC to monitor the effects of particle (chromium, iron, nickel) exposure in welders (Hoffmeyer F et al, J Breath Res 2012;6:027105)
- Cobalt and tungsten in EBC to monitor Co and W exposure (less reliable than urine monitoring) (Broding HC et al, Int Arch Occup Environ Health 2009;82:565-73)

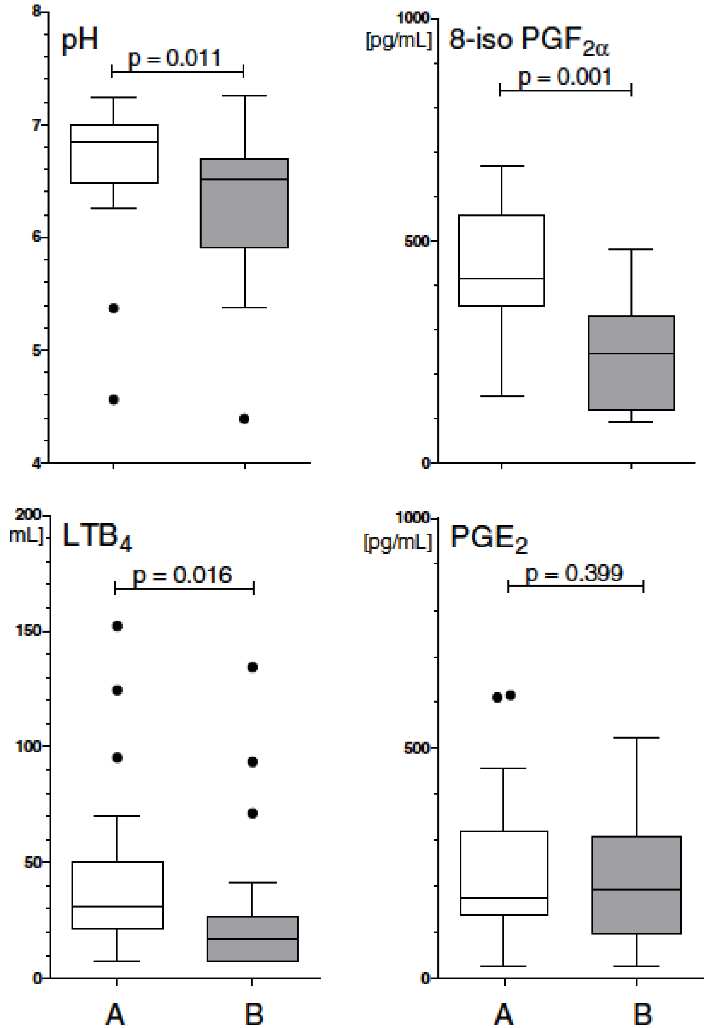
EBC in occupational medicine

- TCDD exposure: EBC 8-IsoP, LTB_4, C_4, D_4, E_4, HNE, MDA, o-Tyr, 8-OHG, 8-OHdG, 5-OHMeU, NO-Tyr (Pelclova D et al. Neuroendocrinol Lett 2011;32, Suppl 1:71-76)
- Asbestosis: EBC 8-IsoP (Pelclova D et al. Ind Health 2008;46:484-9)
- Weld fume exposure: elevated EBC nitrate concentrations (Gube M et al. Int Arch Occup Environ Health 2010;83:803-11)

EBC in occupational medicine

- Grain dust and endotoxin exposure (EBC pH, ammonium, 8-IsoP): acute exposure associated with increased 8-IsoP, chronic exposure associated with decreased ammonium and increased pH (Do R et al. Am J Respir Crit Care Med 2008;178:1048-54)
- Chromium exposure: elevated EBC nitrite concentrations (Murgia N et al. Int J Immunopath Pharmacol 2006;19(4 Suppl):67-71)

EBC analysis in workers exposed to welding fume



exposure in respirable welding fume

Fe = 1.9 µg/m^3

Cr = 1 µg/m^3

Ni = 1 µg/m^3

Median EBC concentrations

Cr < 0.25 µg/L

Fe = 12 µg/L

Ni = 1,12 µg/L

Undetectable EBC Cr 24/43

A = high Fe and Ni, low Cr

B = low Fe and Ni, high Cr

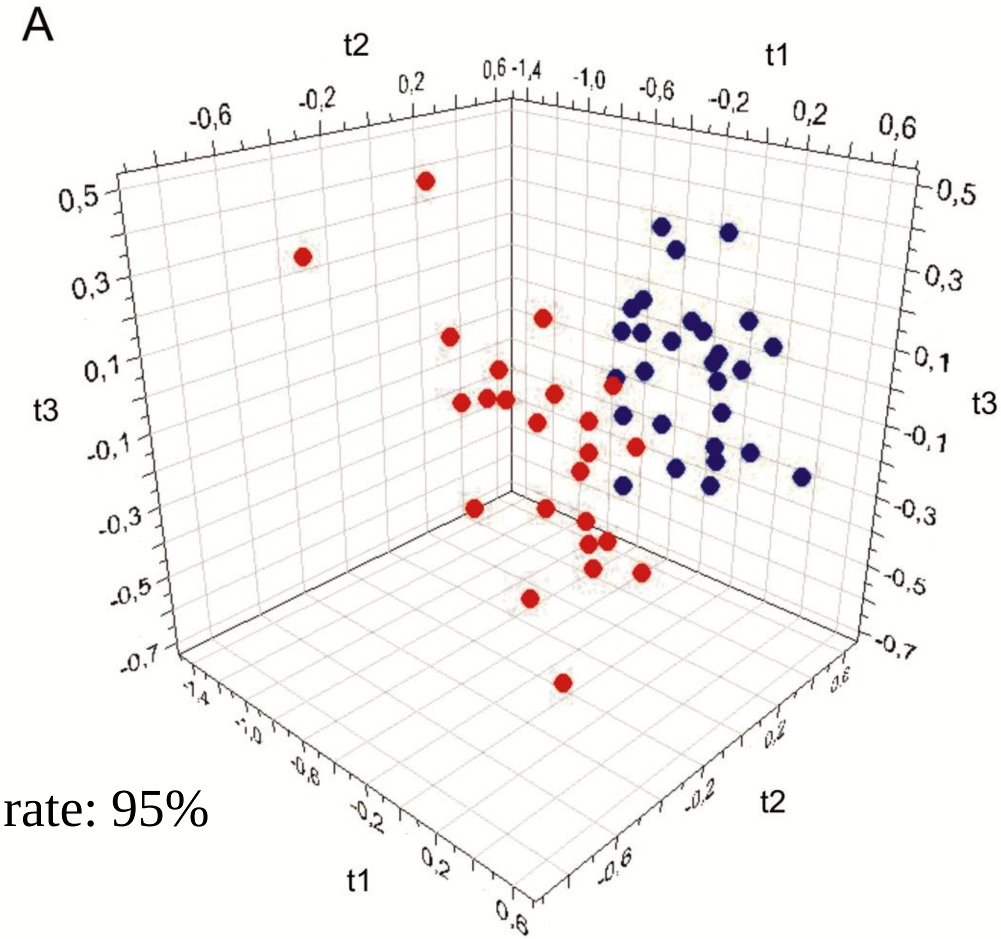
Hoffmeyer F, J Breath Res, 2012

Background

- Metabolomic analysis involves the detection of metabolites in biological samples usually using high resolution Nuclear Magnetic Resonance (NMR) spectroscopy
- NMR spectroscopy enables identification and accurate quantitative assessment of the low-molecular mass endogenous metabolites
- This can be done by comparing the sample spectra to a library of reference spectra derived from standard compounds of known concentrations
- Metabolomics provides a metabolic fingerprint which can be used for classification purposes, and focuses on the most important regions of the NMR spectrum for further analysis

EBC metabolomic analysis: stable CF vs unstable CF

Score Plot of PLS-DA



● Stable CF patients (n = 29)

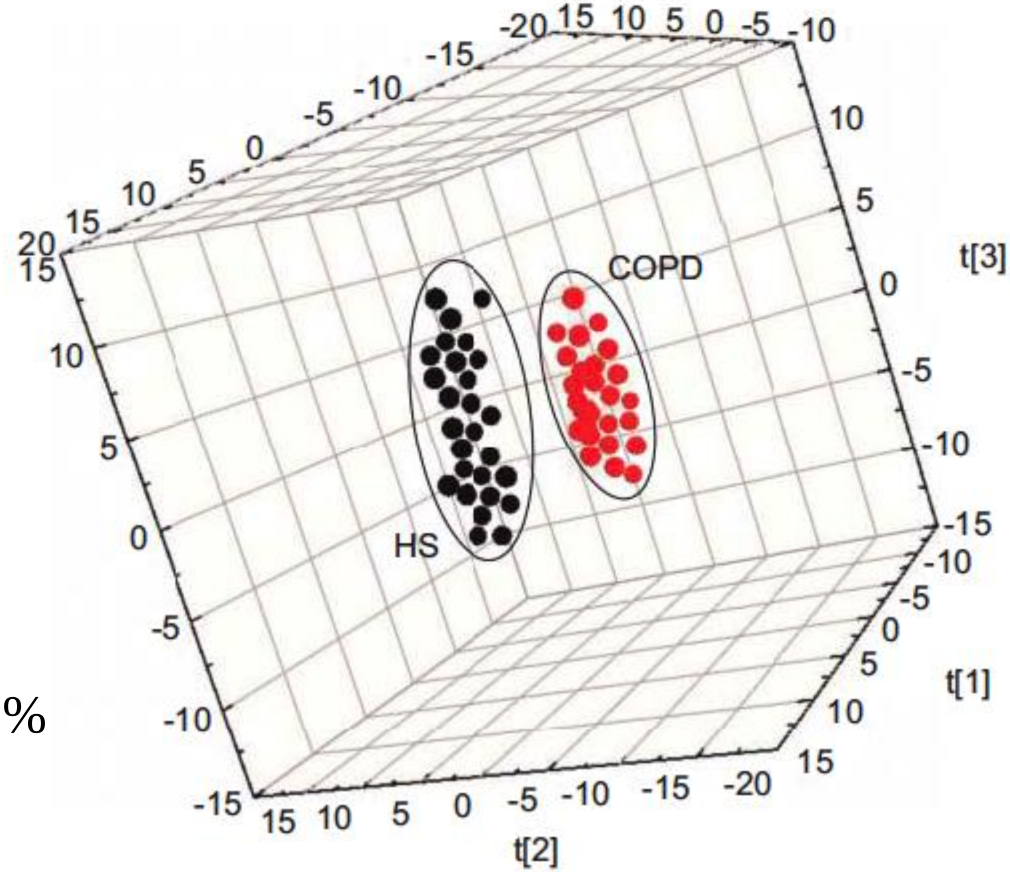
● Unstable CF patients (n = 24)

Classification rate: 95%

Montuschi P. et al., Thorax, 2012

EBC metabolomic analysis in COPD

Score Plot of PLS-DA

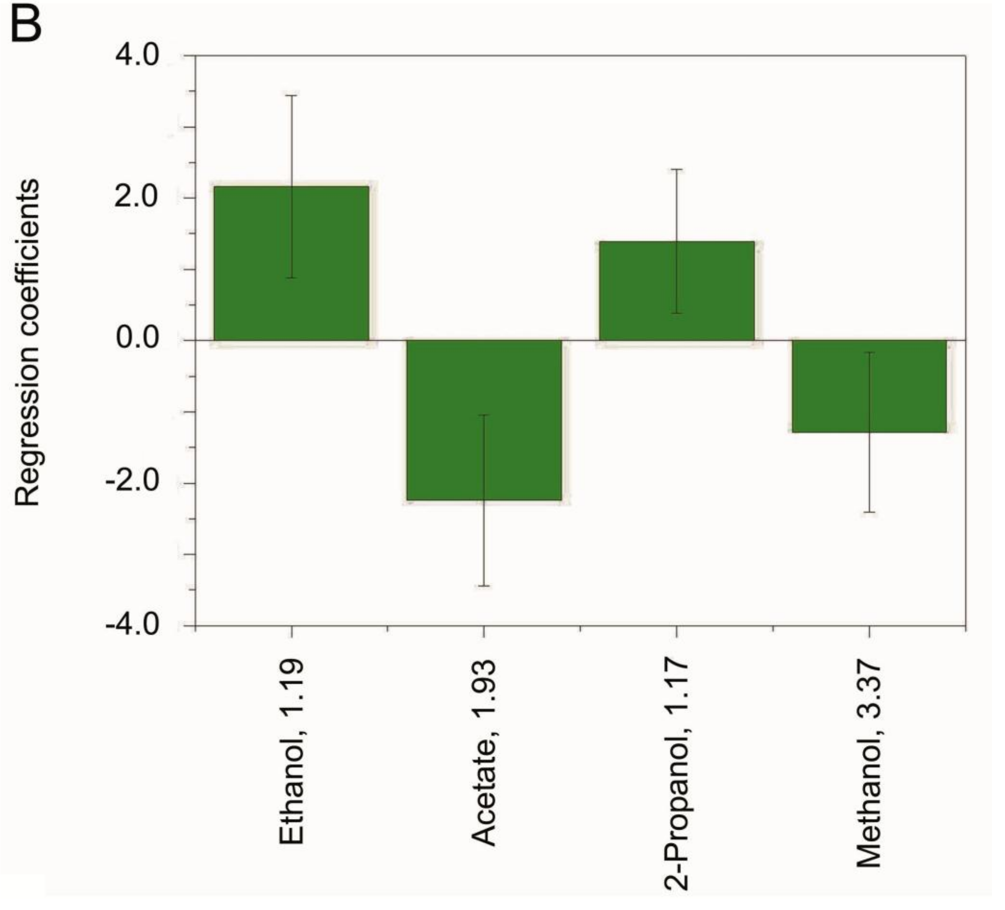


- Healthy subjects (n = 27)
- COPD patients (n = 27)

$R^2 = 99.9\%$

Montuschi P. et al., Eur.Respir. J., 2012

Stable vs Unstable CF Patients: a model based on 4 selected metabolites



$R^2 = 0.80$
$Q^2 = 0.65$

Montuschi P. et al., Thorax, 2012

Electronic nose: basic principles

- Electronic nose is an array of different individual chemical sensors, but globally selective according to the principle that each sensor senses more compounds and each compound is sensed by more sensors
- Electronic nose strategy is based on the instrument sensitivity to the totality of the compounds, rather than to a specific one

Potential applications of e-nose in respiratory medicine

- Asthma
- COPD
- Lung cancer
- Healthy smokers

E-nose: smellprints

- The steady state frequency or resistance shifts of the electronic nose sensors give rise to a pattern (“smellprint”), and a collection of measurements produces a set of patterns that is analyzed by a pattern recognition algorithm for classification purposes

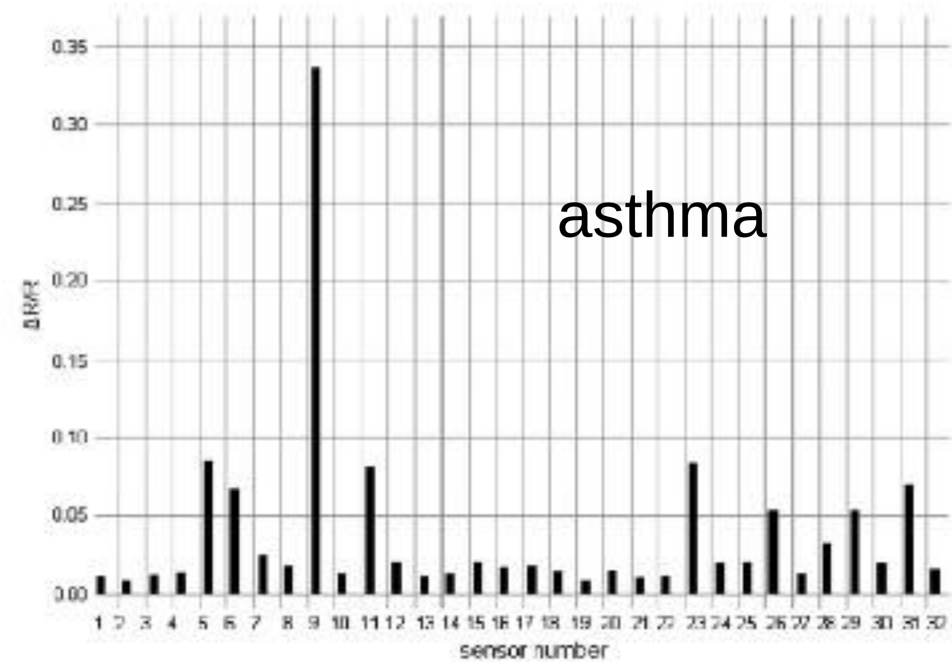
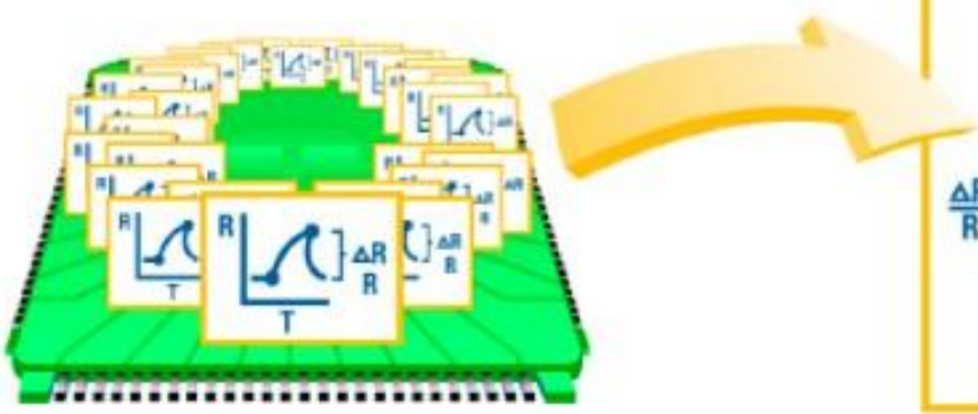
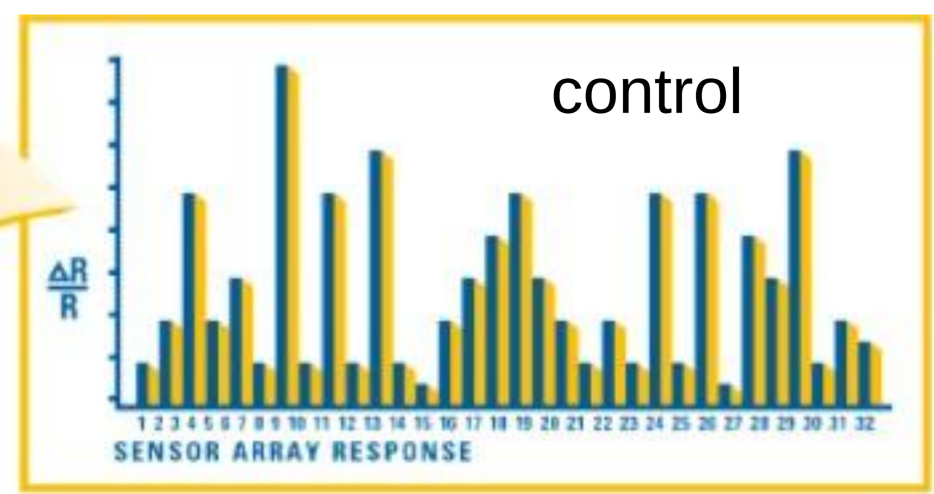


FIG 1. Example of pattern of relative differential electrical resistance (ΔR/R) of an array of 32 polymer sensors of the electronic nose, which represents the smellprint of a VOC mixture in exhaled breath of a single volunteer with asthma.





Electronic nose Cyranose 320®

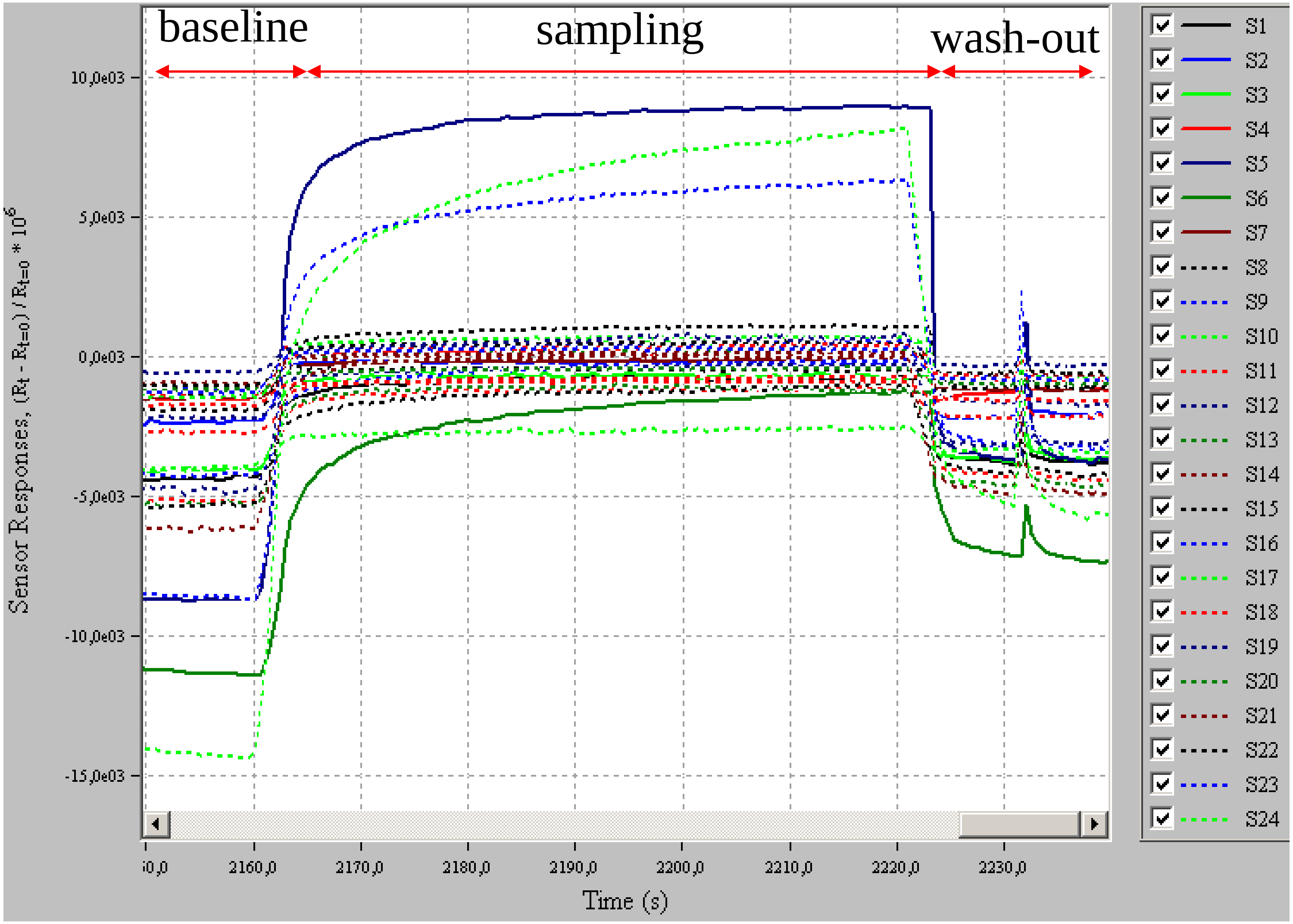
- consists of 32 organic polymer sensor array
- detects VOCs in the ppb range
- VOC mixtures bind reversibly to sensors depending on:
    - molecular size
    - molecular shape
    - dipole moment
    - hydrogen binding capacity

Lewis NS. Acc Chem Res 2004;37:663-672

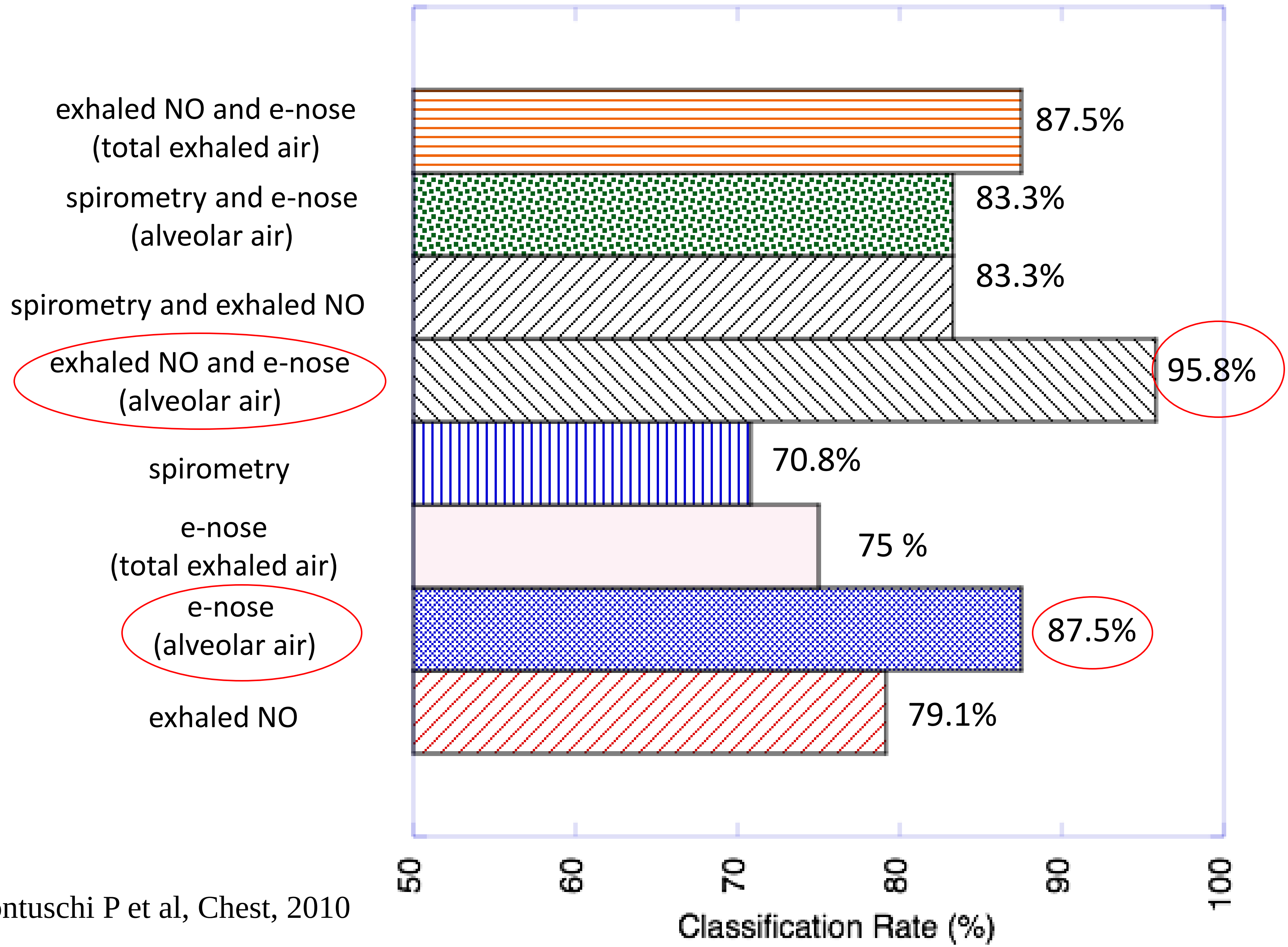
Collection of breath samples

- 5 min tidal breathing with VOC-filtered air (wash-in)
- A single expiratory VC (0.25-0.5 l/s)
- Analysing breath samples within 30 min

Cyranose 320: sample response



Diagnostic performance of e-nose, eNO and PFT



Montuschi P et al, Chest, 2010

2008 Innovative Medicine Initiatives (IMI) call topic: Understanding Severe Asthma

Applicant Consortium:

Unbiased Biomarkers for the Prediction of Respiratory Disease Outcomes (U-BIOPRED)

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Omics technologies

LIPIDOMICS	
Lipidomic screen by NanoMate-ESI	rapid profiling of *m/z* signatures
Lipidomic screen by LC/QTOF	More detailed lipid profiling by LC and m/z signatures
Shotgun lipidomics	Diagnostic precursor and neutral loss scan - looking at particfular classes of lipids
MRM assays	specific oxidised lipids
GC/MS MRM	eicosanoids and endocannabinoids
BREATHOMICS	
eNose	Smellprints (profiles) of volatile organic volatile compounds (VOCs) mixtures
GC/MS	Identities of individual VOCs and mixtures
High-resolution proton nuclear magnetic resonance (NMR) spectroscopy	metabolite profiling in exhaled breath condensate

Breathomics: potential implications for respiratory and occupational medicine

- In combination with other omics platforms and techniques, breathomics could contribute to the identification of subphenotypes of patients with respiratory diseases and a better charactrization of single patients
- This integrated strategy might lead to:
  - Identification of new biomarkers and/or surrogate markers for respiratory disease
  - A tailored pharmacological treatment
  - Development of new drugs
- EBC analysis in occupational research provides a tool for the simultaneous monitoring of chemical exposure and target effects
- Breathomics might be suitable for identifying those subjects at elevated risk for developing respiratory disease

F_ENO: disadvantages

- NO analyzers are relatively expensive
- Technology not widely available
- Single breath measurement is difficult in preschool children
- Poor correlation with PFT and AHR after steroids
- Only measures one component of inflammatory response
- Does not correlate with asthma severity (NHLBI)
- Concentrations are affected by several factors, including atopy and gene polymorphisms
- The dose response to ICS is steep and plateaus early

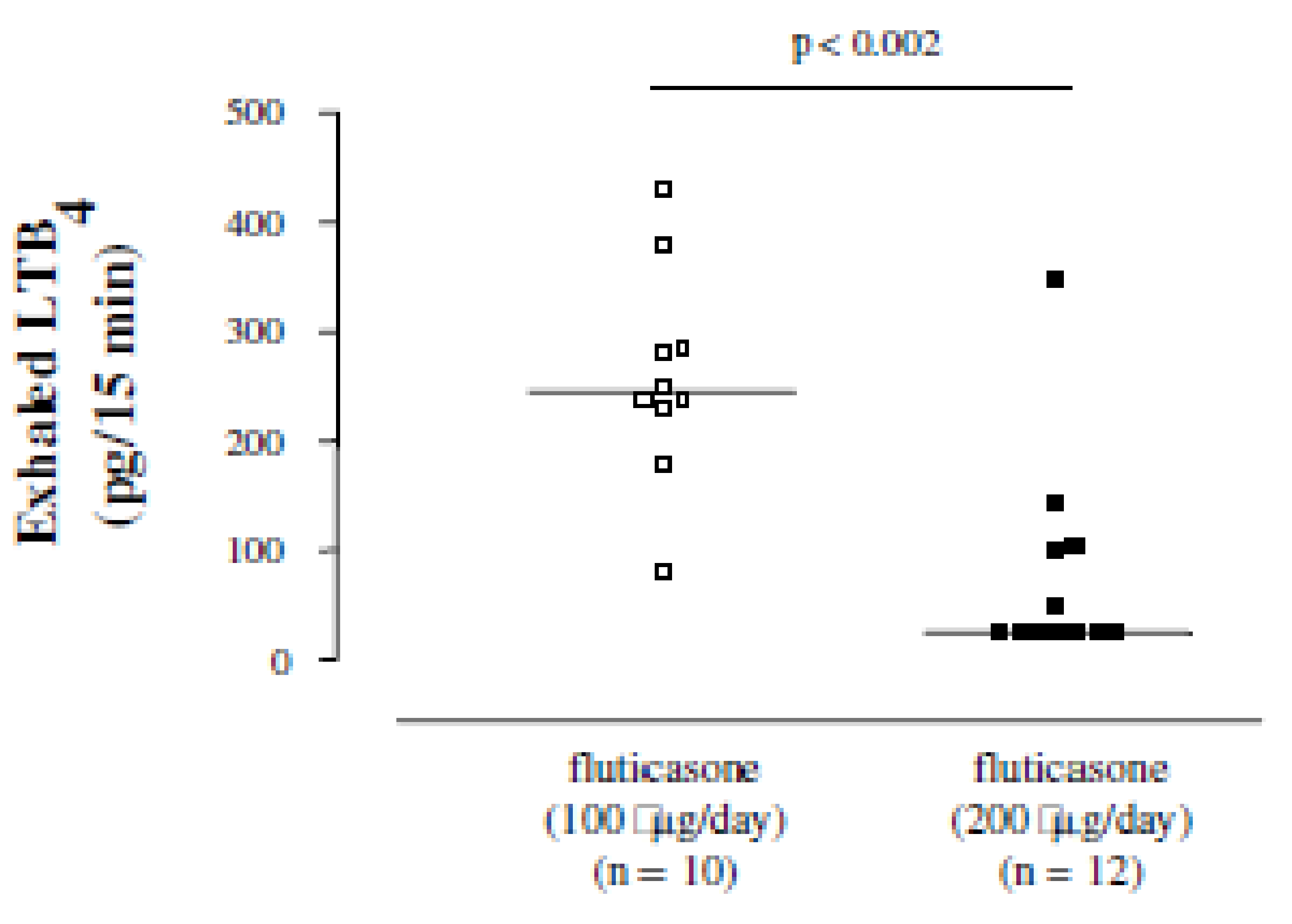
Bates C.A. and Silkoff P.E., JACI, 2003

Measurement of F_ENO in occupational medicine

- Exposure to:
  - organic dust (Von Essen SG et al, 1998)
  - wood dust (Obata H et al,1999)
  - wood smoke (Stockfeld et al, 2012)
  - ozone (Olin AC et al, 2004)
  - latex (Baur X et al, 2005)
  - isotiocyanates (Barbinova L et al, 2006)
- Occupational asthma (Lemiere C et al, 2007)
  - bakers/pastry makers, hairdressers (Tossa P et al, 2010)
  - farmers after specific inhalation test (Swierczynska-Machura D et al, 2008; Dressel H et al, 2007)

Asbestosis (Lehtonen C et al, 2007)

LC/MS/MS of LTB_4 in EBC in asthmatic children



Montuschi P. et al., Respir. Res., 2005