Convegno Nazionale GISCoR 2015

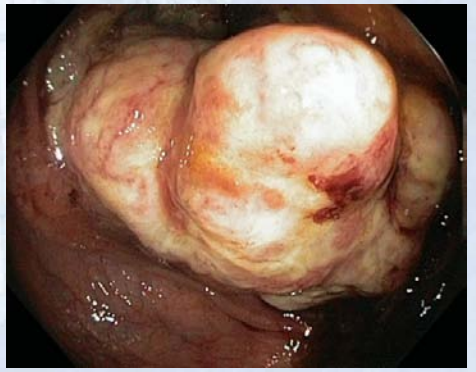
Screening per il cancro colorettale. Prospettive future: il ruolo dei VOCs

Prof Donato F Altomare, MD
Dept of Emergency and Organ Transplantation,
University of Bari, Italy

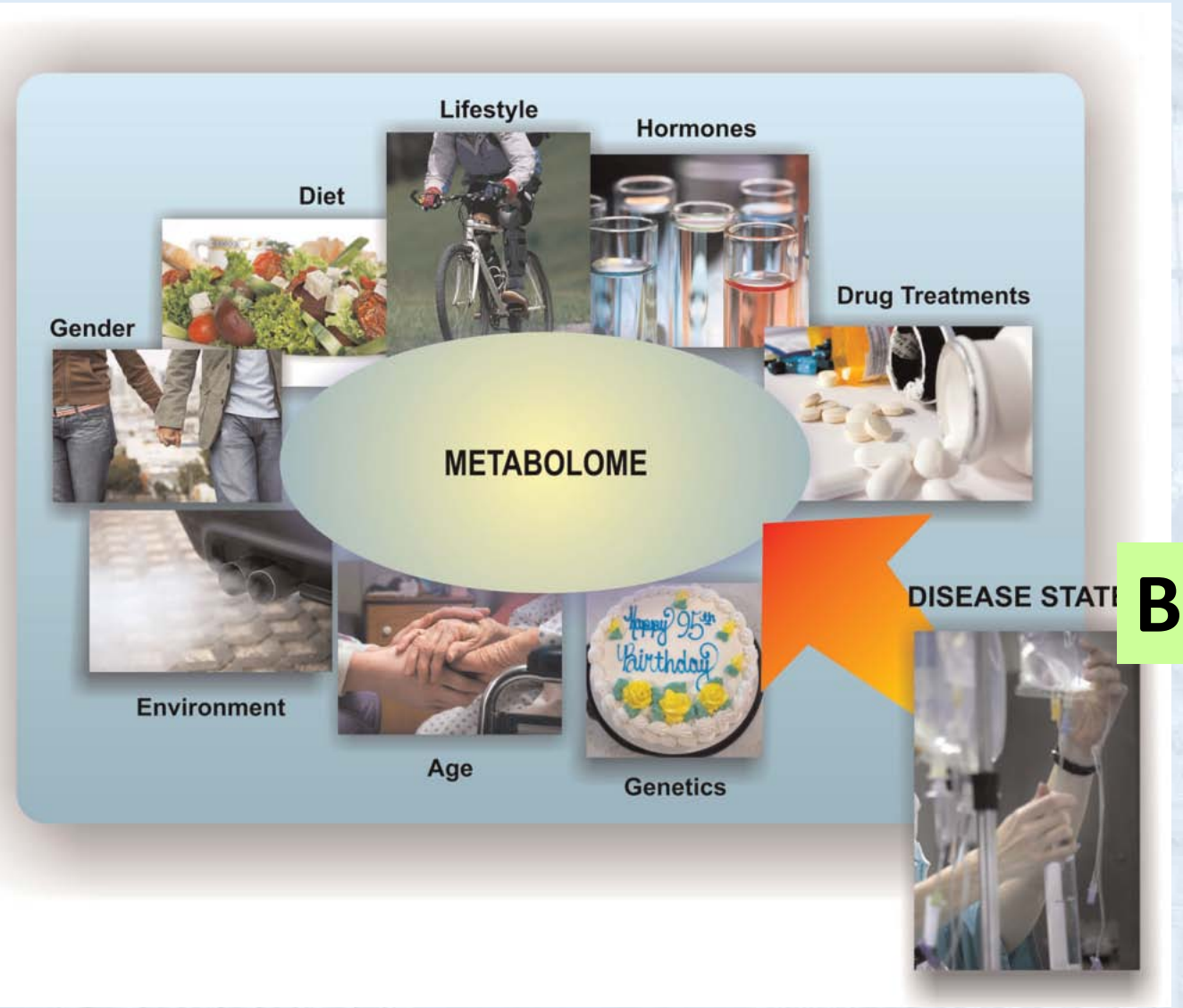
19 -20 NOVEMBRE 2015
NAPOLI | Hotel Royal Continental

Colorectal cancer (CRC) screening

- CRC screening is cost/effective particularly in countries with financial resourses
- Colonoscopic screening is limited by its cost and invasiveness while FOBT/FIT have poor patient's compliance and reliability.
- A novel, non-invasive, highly sensitive biomarkers to improve the detection of CRC is strongly awaited.



Metabolomics



Breathomics

Exhaled Volatile Organic Compounds (VOCs)
first application in human

Proc. Nat. Acad. Sci. USA
Vol. 68, No. 10, pp. 2374–2376, October 1971

Quantitative Analysis of Urine Vapor and Breath by Gas–Liquid Partition Chromatography

(orthomolecular medicine/vitamins/controlled diet)

LINUS PAULING*, ARTHUR B. ROBINSON*, ROY TERANISHI†, AND PAUL CARY*

* Department of Chemistry, Stanford University, Stanford, California 94305; and † Western Regional Laboratory, U.S. Department of Agriculture

Contributed by Linus Pauling, July 29, 1971

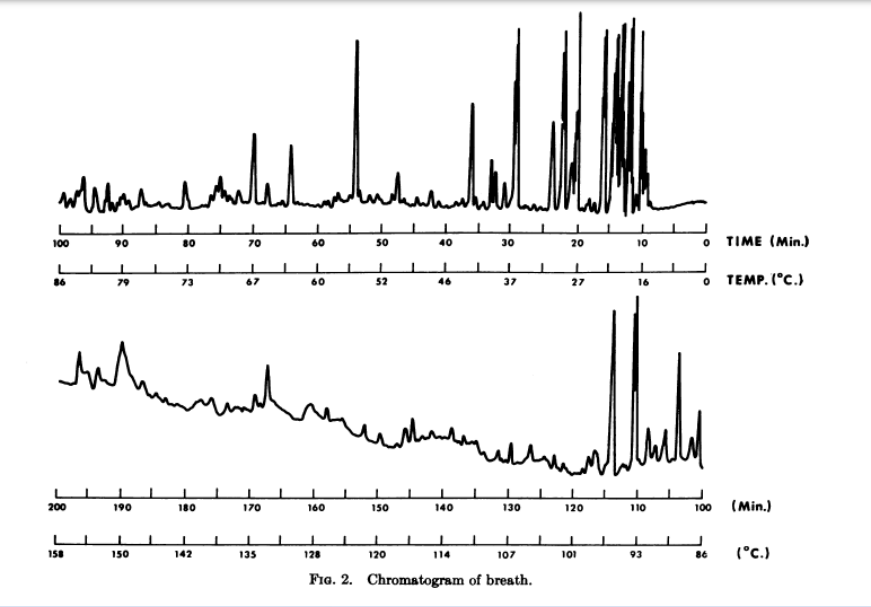
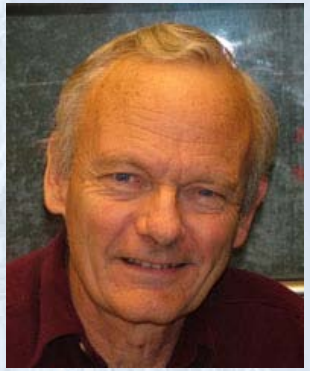


FIG. 2. Chromatogram of breath.

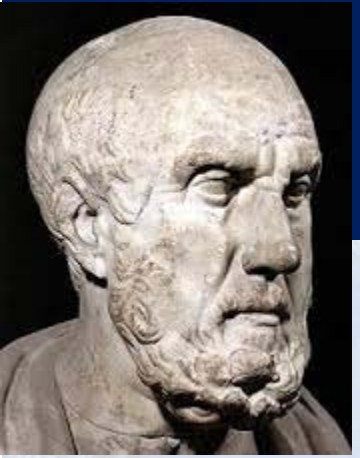
Linus Pauling



Arthur Robinson

New options for colorectal cancer screening:
VOCs (organic Volatile Compounds) analysis

- **VOCs in the headspace of blood**
- **VOCs in the headspace of urine**
- **VOCs in the headspace of feces**
- **VOCs in the exhaled breath**



Breath features and human diseases

- 460 BC, Ippocrates: “fetor oris” and “fetor hepaticus”.
- 1874 England Anstie: ethanol in the breath of drinkers
- 1897 Nebelthau & Geelmuyden: acetone in the breath of diabetic patients.

Even without knowing the chemicals our olfactory receptors are able to identify 4000-10000 different smells

Volatile Organic Compounds in the breath reflect the health status of the patient like a «*breathprint*»

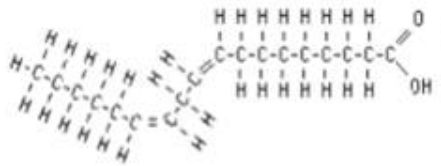
VOCs and Human Cancer

Tumor growth

Gene/protein changes

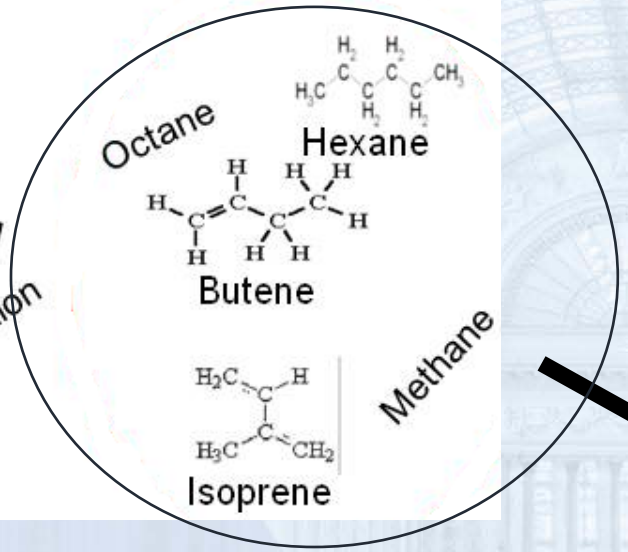
Oxidative stress

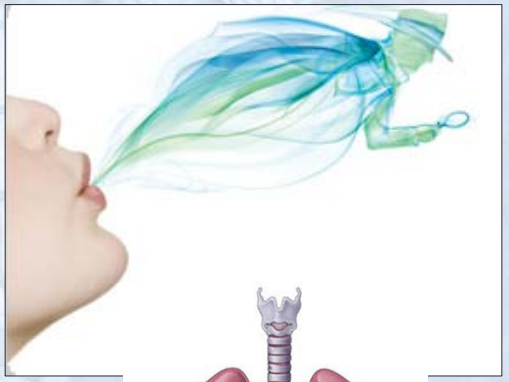
Polyunsaturated fatty acids (PUFAs)

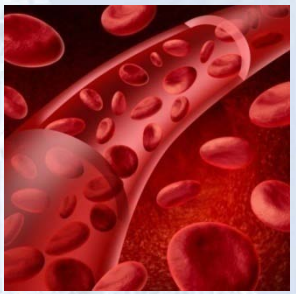


O_2 O_2

Lipid peroxidation

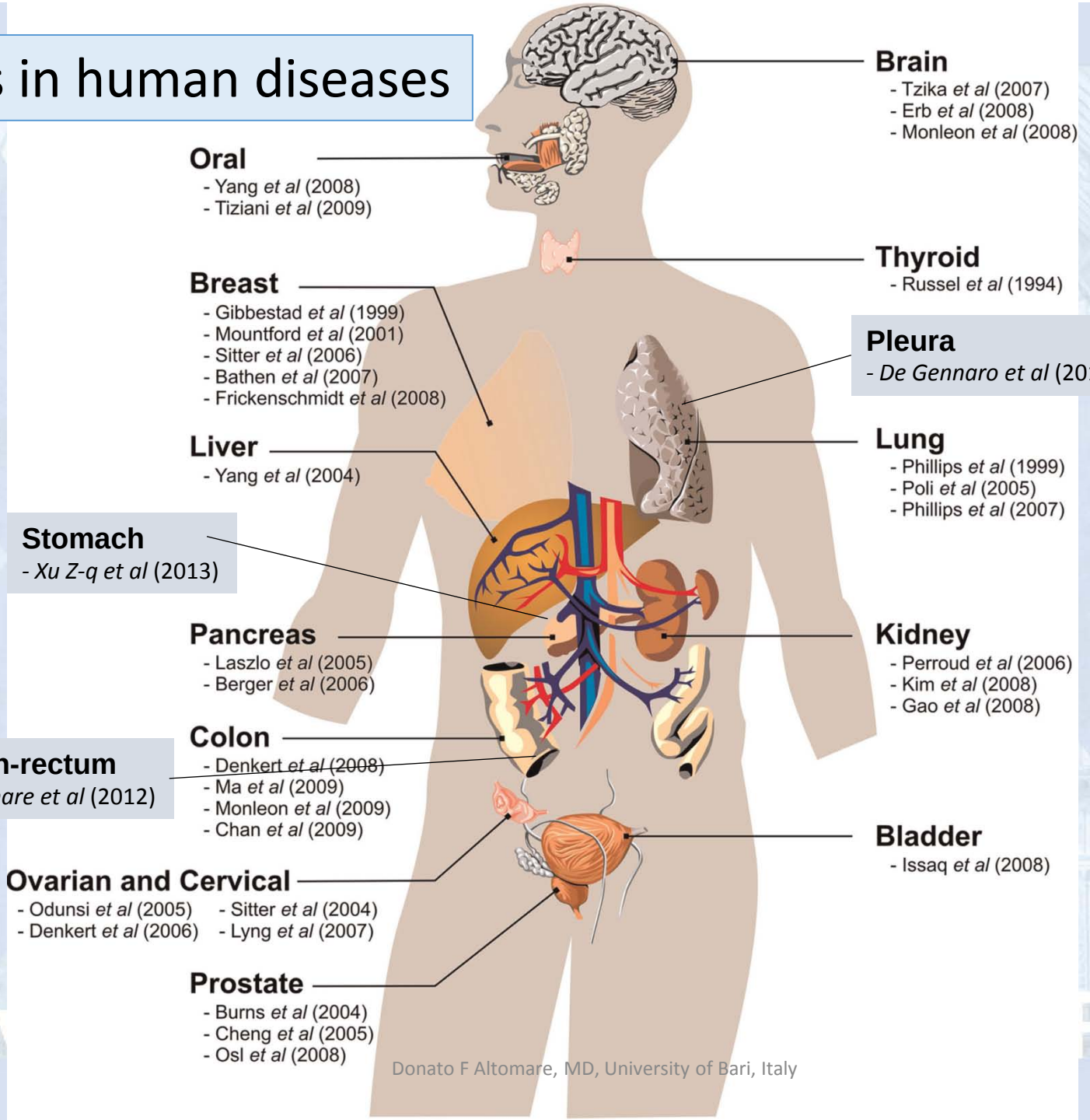






Irrespectively of the site of production the VOCs are reversed into the blood stream and reach the lung were they can be exhaled

VOCs in human diseases



Brain
- Tzika *et al* (2007)
- Erb *et al* (2008)
- Monleon *et al* (2008)

Oral
- Yang *et al* (2008)
- Tiziani *et al* (2009)

Thyroid
- Russel *et al* (1994)

Breast
- Gibbestad *et al* (1999)
- Mountford *et al* (2001)
- Sitter *et al* (2006)
- Bathen *et al* (2007)
- Frickenschmidt *et al* (2008)

Pleura
- *De Gennaro et al* (2010)

Liver
- Yang *et al* (2004)

Lung
- Phillips *et al* (1999)
- Poli *et al* (2005)
- Phillips *et al* (2007)

Stomach
- *Xu Z-q et al* (2013)

Pancreas
- Laszlo *et al* (2005)
- Berger *et al* (2006)

Kidney
- Perroud *et al* (2006)
- Kim *et al* (2008)
- Gao *et al* (2008)

Colon
- ~~Denkert *et al* (2008)~~
- Ma *et al* (2009)
- Monleon *et al* (2009)
- Chan *et al* (2009)

Colon-rectum
- *Altomare et al* (2012)

Bladder
- Issaq *et al* (2008)

Ovarian and Cervical
- Odunsi *et al* (2005)
- Denkert *et al* (2006)
- Sitter *et al* (2004)
- Lyng *et al* (2007)

Prostate
- Burns *et al* (2004)
- Cheng *et al* (2005)
- Osl *et al* (2008)

Breathomics in Colorectal Cancer

Br J Surg. 2013 Jan;100(1):144-50

Original article

Exhaled volatile organic compounds identify patients with colorectal cancer

D. F. Altomare[1], M. Di Lena[1], F. Porcelli[2], L. Trizio[2], E. Travaglio[1], M. Tutino[2], S. Dragonieri[3], V. Memeo[1] and G. de Gennaro[2]

Departments of [1]Emergency and Organ Transplantation, [2]Chemistry and [3]Lung Diseases, University 'Aldo Moro' of Bari, Bari, Italy
Correspondence to: Professor D. F. Altomare, Department of Emergency and Organ Transplantation, c/o Azienda Ospedaliero-Universitaria Policlinico Bari, Piazza G. Cesare, 11-70124 Bari, Italy (e-mail: donatofrancesco.altomare@uniba.it)

la Repubblica.it
il mondo in diretta
24 ore su 24

CNN

BBC

VOCs in COLORECTAL CANCER *(Altomare DF et al Br J Surg 2013)*

Design of the study

Patients with Colorectal Cancer

Healthy controls (negative colonoscopy)

Breath test
GC-MS analysis
Histology

Breath test
GC-MS analysis

37

Training set: contruction of a predictive model by PNN

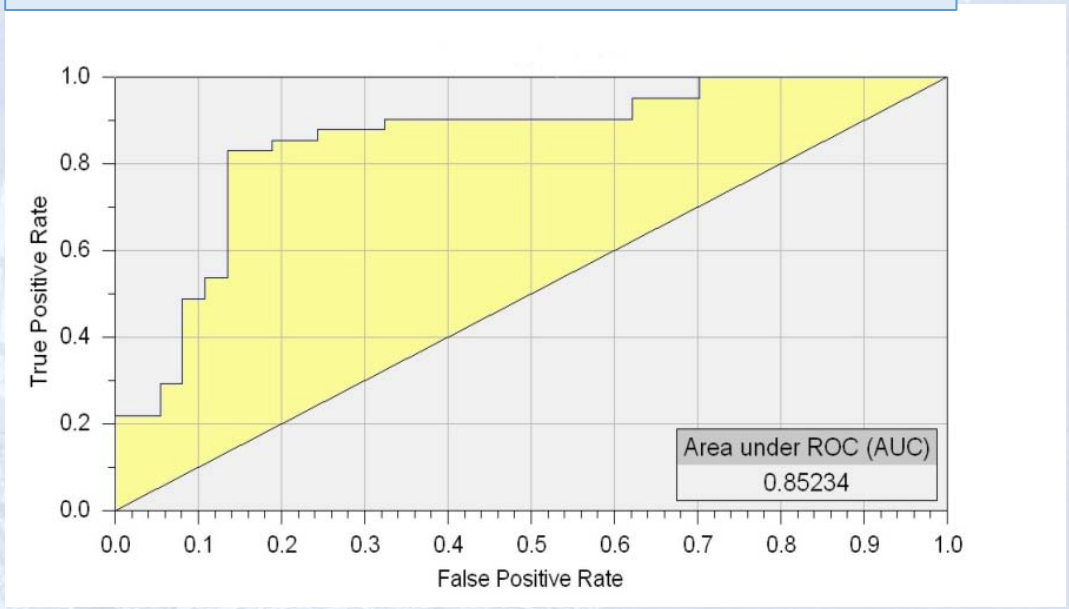
41

25

Validation trial:
Test the predictive model

VOCs in COLORECTAL CANCER

15 volatile organic compounds	Recognition capabilities (%)
Nonanal	80
4-methyl-2-pentanone	88
Decanal	92
2-methylbutane	87
1,2-pentadiene	95
2-methylpentane	87
3-methylpentane	87
methylcyclopentane	80
Cyclohexane	94
Methylcyclohexane	92
1,3-dimethylbenzene	96
4-methyloctane	80
1,4-dimethylbenzene	80
A (4-methyl-undecane, RT=11·3)	59
B (trimethy-ldecane, RT=13·2)	72



Accuracy	**85,5%**
Sensitivity	**83,3%**
Specificity	**87,9%**

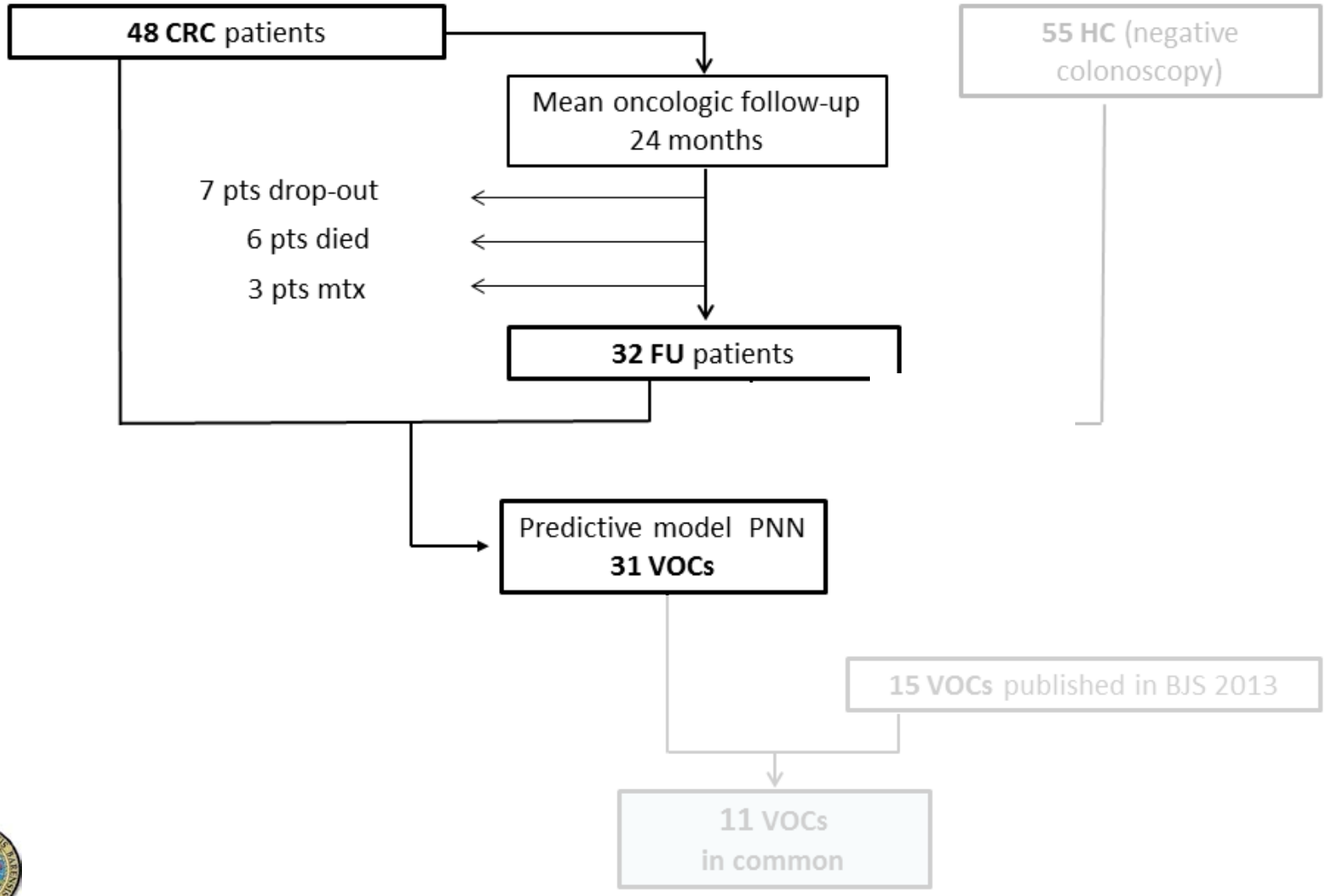
Effects of Curative Colorectal Cancer Surgery on Exhaled Volatile Organic Compounds and Potential Implications in Clinical Follow-up

Donato F. Altomare, MD, Maria Di Lena, MD, Francesca Porcelli, PhD,y Elisabetta Travaglio, MD, Francesco Longobardi, PhD,y Maria Tutino, PhD,y Norma Depalma, MD, Giuseppina Tedesco, BS,y Annamaria Sardaro, BS,y Riccardo Memeo, PhD, and Gianluigi de Gennaro, PhDy

Annals of Surgery Volume 262, Number 5, November 2015

To evaluate whether the VOCs pattern in CRC patients could be modified by curative surgery and eventually used in the oncologic follow up.

Design of the study 1.
VOCs analysis before and after curative surgery

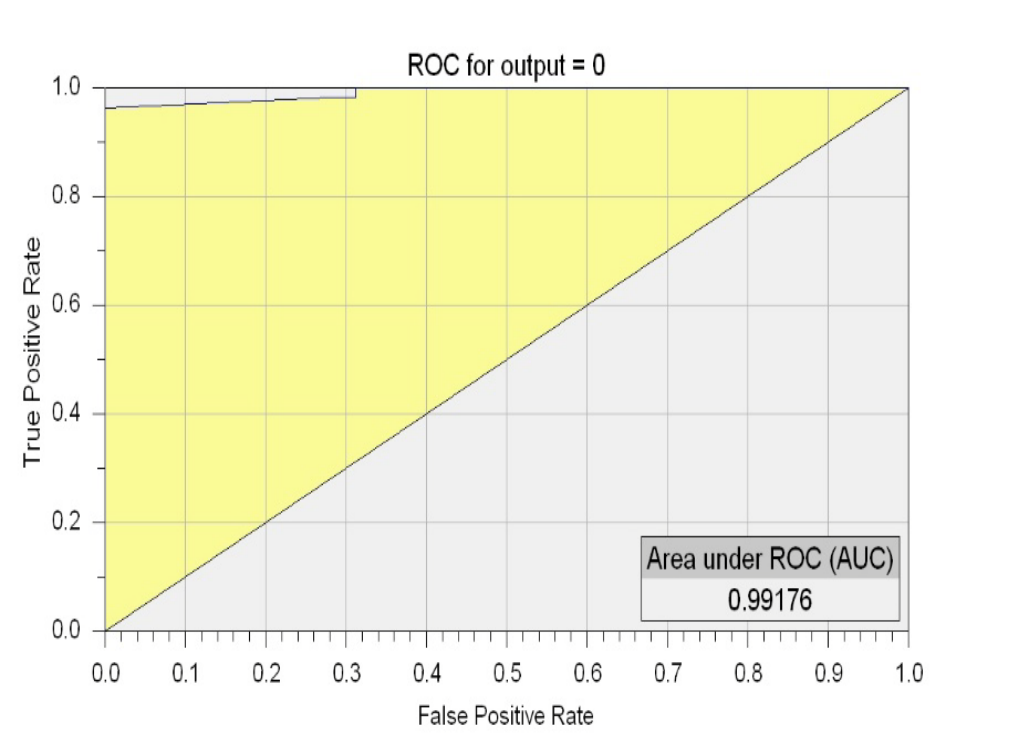


31 VOCs	
1,2-pentadiene	beta-pinene
2-methylbutane	1-methyl-3- (1-methylethyl)benzene
2-methylpentane	X*
1-(1-methylethenyl)-2-(1-methylethyl)benzene	5-butylnonane
methylcyclopentane	undecane
cyclohexane	Y*
heptane	nonanal
methylcyclohexane	dodecane
4-methyl-2-pentanone	decanal
1-methylnaphthalene	1-ethyl-1,2,4-trimethylbenzene
1-octene	1-ethyl-2,4,5-trimethylbenzene
octane	2,3-dihydro-1,6-dimethyl-1H-Indene
1,2,3-trimethylbenzene	2,3-dihydro-4,7-dimethyl-1H-Indene
1,3-dimethylbenzene	1,3-dimethyl-5-(1-methylethyl)benzene
1,4-dimethylbenzene	2-methylnaphthalene
propylbenzene	

CRC patients vs disease-free patients in follow-up

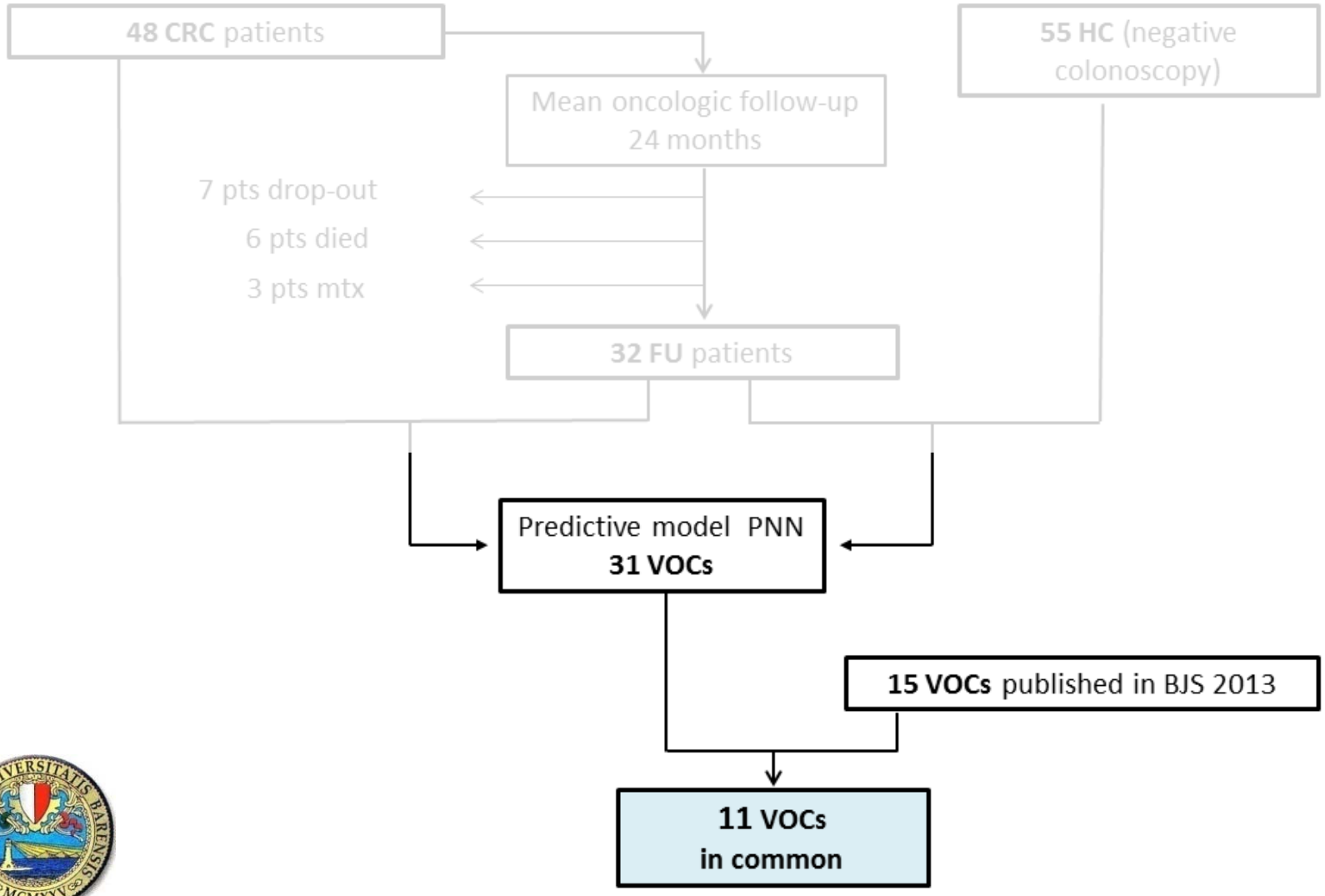
PNN analysis

SENSITIVITY	**100 %**
SPECIFICITY	**95.83%**
ACCURACY	97.50 %
AUC	0.992



Design of the study 3.

VOCs in common with our previous study *(Br J Surg 2013)*



15 VOCs (Previous study)
nonanal
4-methyl-2-pentanone
decanal
2-methylbutane
1,2-pentadiene
2-methylpentane
3-methylpentane
methylcyclopentane
cyclohexane
Methylcyclohexane
1,3-dimethylbenzene
4-methyloctane
1,4-dimethylbenzene
A (4-methyl-undecane, RT=11·3)
B (trimethy-ldecane, RT=13·2)

11 Common VOCs

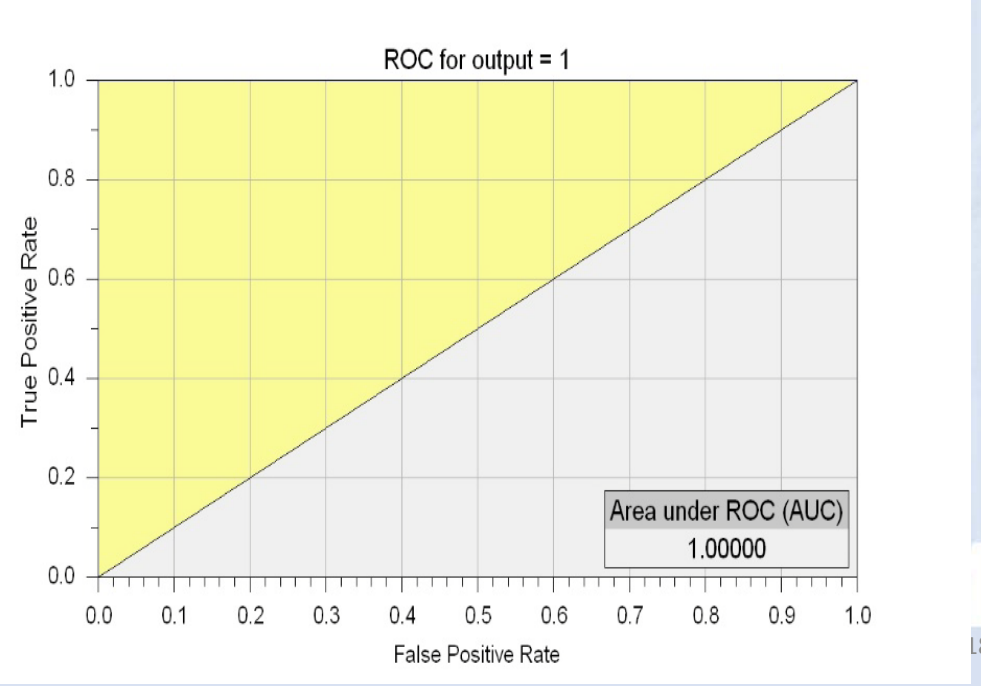
31 VOCs (New study)	
1,2-pentadiene	beta-pinene
2-methylbutane	1-methyl-3- (1-methylethyl)benzene
2-methylpentane	X*
1-(1-methylethenyl)-2-(1-methylethyl)benzene	5-butylnonane
methylcyclopentane	undecane
cyclohexane	Y*
heptane	**nonanal**
methylcyclohexane	dodecane
4-methyl-2-pentanone	**decanal**
1-methylnaphthalene	1-ethyl-1,2,4-trimethylbenzene
1-octene	1-ethyl-2,4,5-trimethylbenzene
octane	2,3-dihydro-1,6-dimethyl-1H-Indene
1,2,3-trimethylbenzene	2,3-dihydro-4,7-dimethyl-1H-Indene
1,3-dimethylbenzene	1,3-dimethyl-5-(1-methylethyl)benzene
1,4-dimethylbenzene	2-methylnaphthalene
propylbenzene	

VOCs identified in common with our previous study *(Br J Surg 2013)*

11 VOCs
1,2-pentadiene
2-methylbutane
3-methylpentane
methylcyclopentane
cyclohexane
decanal
nonanal
methylcyclohexane
4-methyl-2-pentanone
1,4-dimethylbenzene
1,3-dimethylbenzene

CRC patients vs disease free patients in follow-up

SENSITIVITY	**100 %**
SPECIFICITY	**97.92%**
ACCURACY	98.75 %
AUC	1



Comments

- The VOCs pattern from CRC patients is clearly ***modified by cancer removal*** confirming the tight relationship between cancer metabolism and exhaled VOCs.
- Vocs analysis seems to have a high reliability to ***identify patients disease-free after curative CRC resection***.

What makes the use of VOCs analysis still far away from routine application in colorectal cancer screening

- **Biological material used**
- Analytical platforms
- Sampling devices
- Identification of the pattern of VOCs involved
  - Testing the specificity of the VOCs pattern for this diseases compared to intestinal polyps, IBD
  - Testing the reliability of the test compared to FIT
- Assessment of an easy and portable device for online result analysis
  - E nose
  - QEPAS
  - Odour-binding Proteins

Biological materials and Volatile Organic Compounds

- Headspace of blood
- Headspace of Urine
- Headspace of Feces
- Exhaled breath

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Analytical platforms used

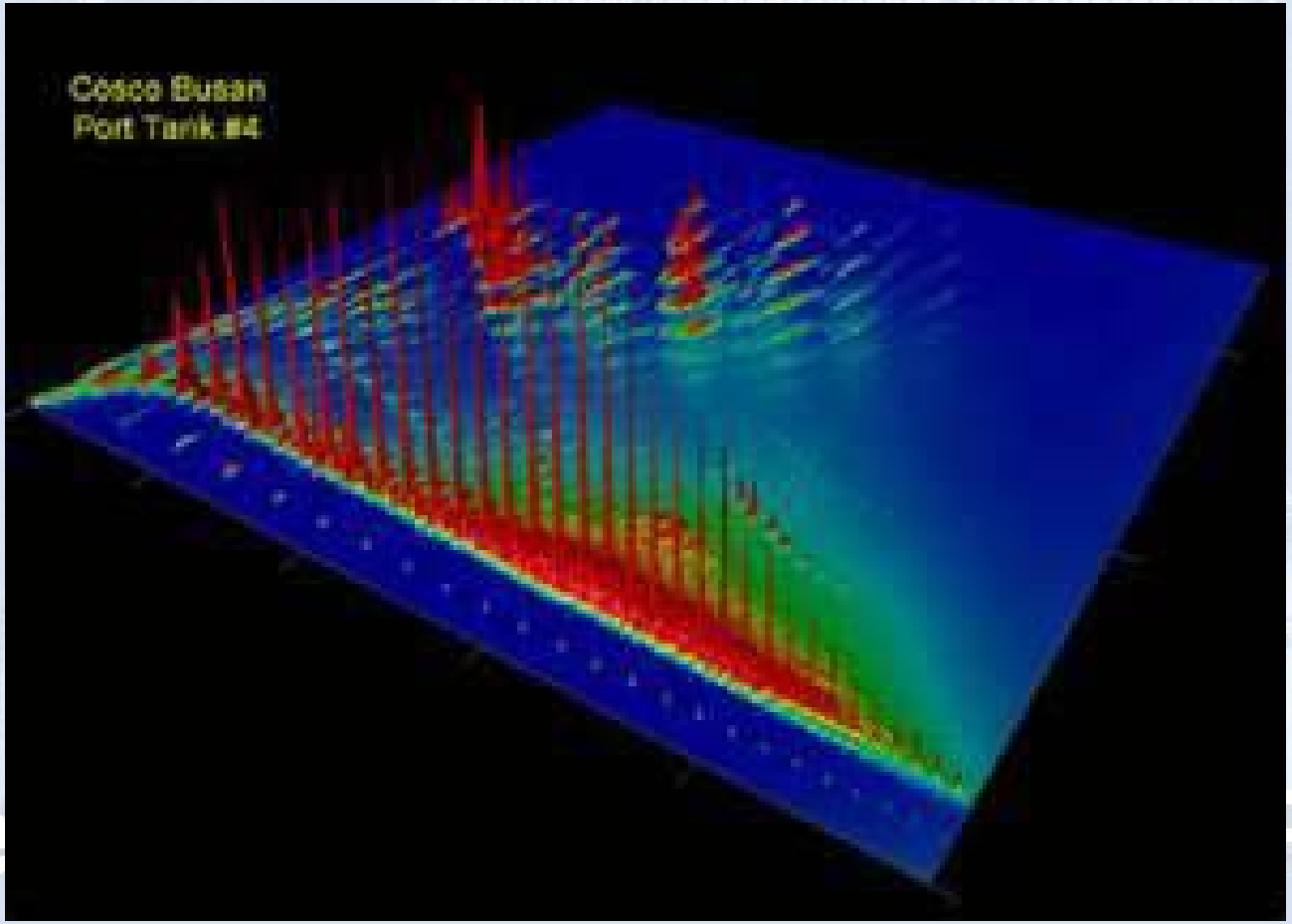
- *Gas chromatography-mass spectrometry (GC-MS) (7 studies)*
- *Field Asymmetric Ion Mobility Spectrometer* (FAIMS) (1 paper on Urinary VOCs)
- *Selected Ion Flow Tube Mass Spectrometry (SIFT-MS) (1 study on fecal VOCs)*
- *Electronic-nose (E-nose)*

Gas Chromatograph/ Mass spectrometer

New Analytical platforms

Two dimension Gas Chromatography

Increases the number of detectable VOCs



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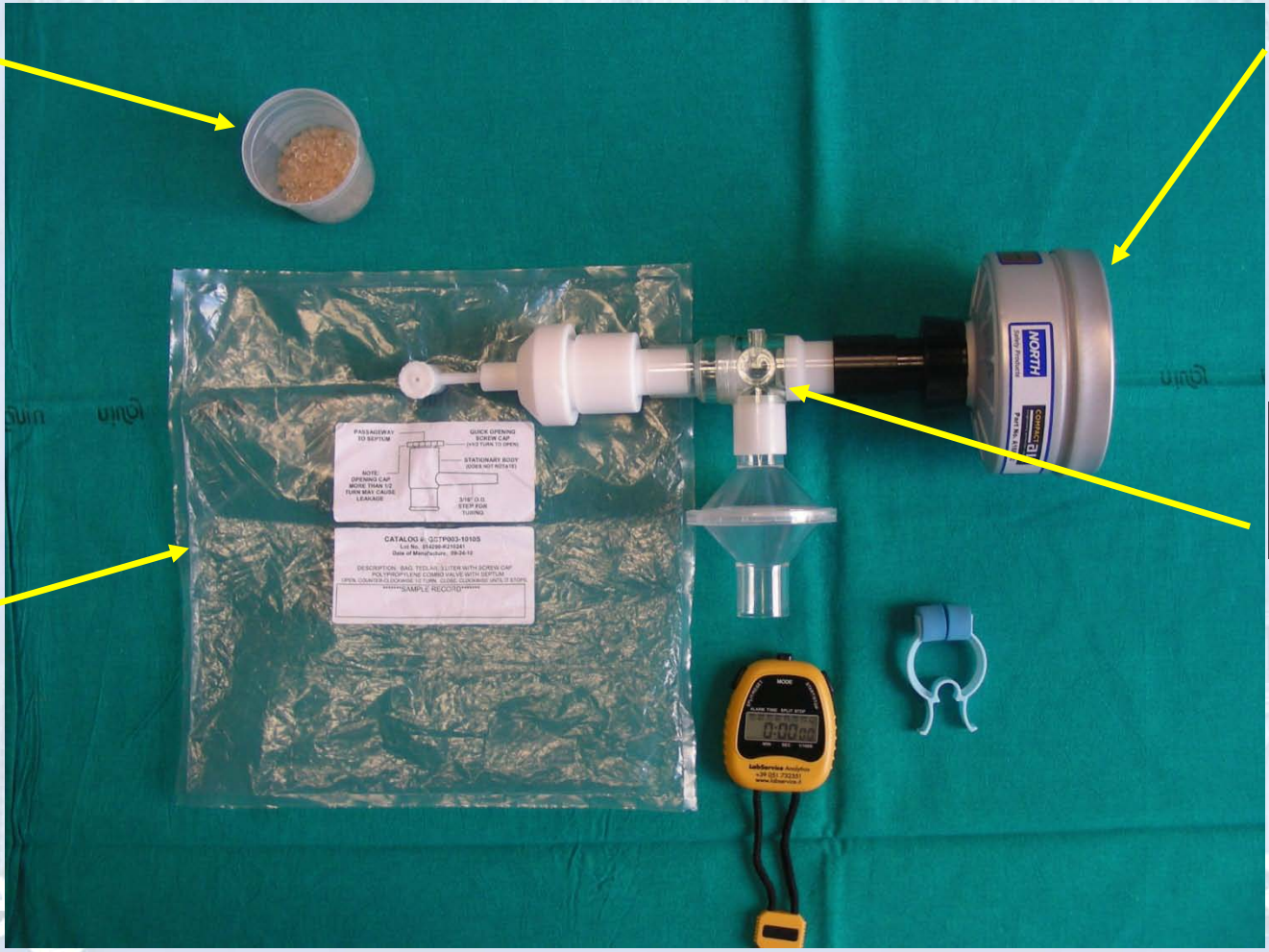
Breath sampling device.

Donato F Altomare, MD, University of Bari, Italy

environment VOCs

Filter

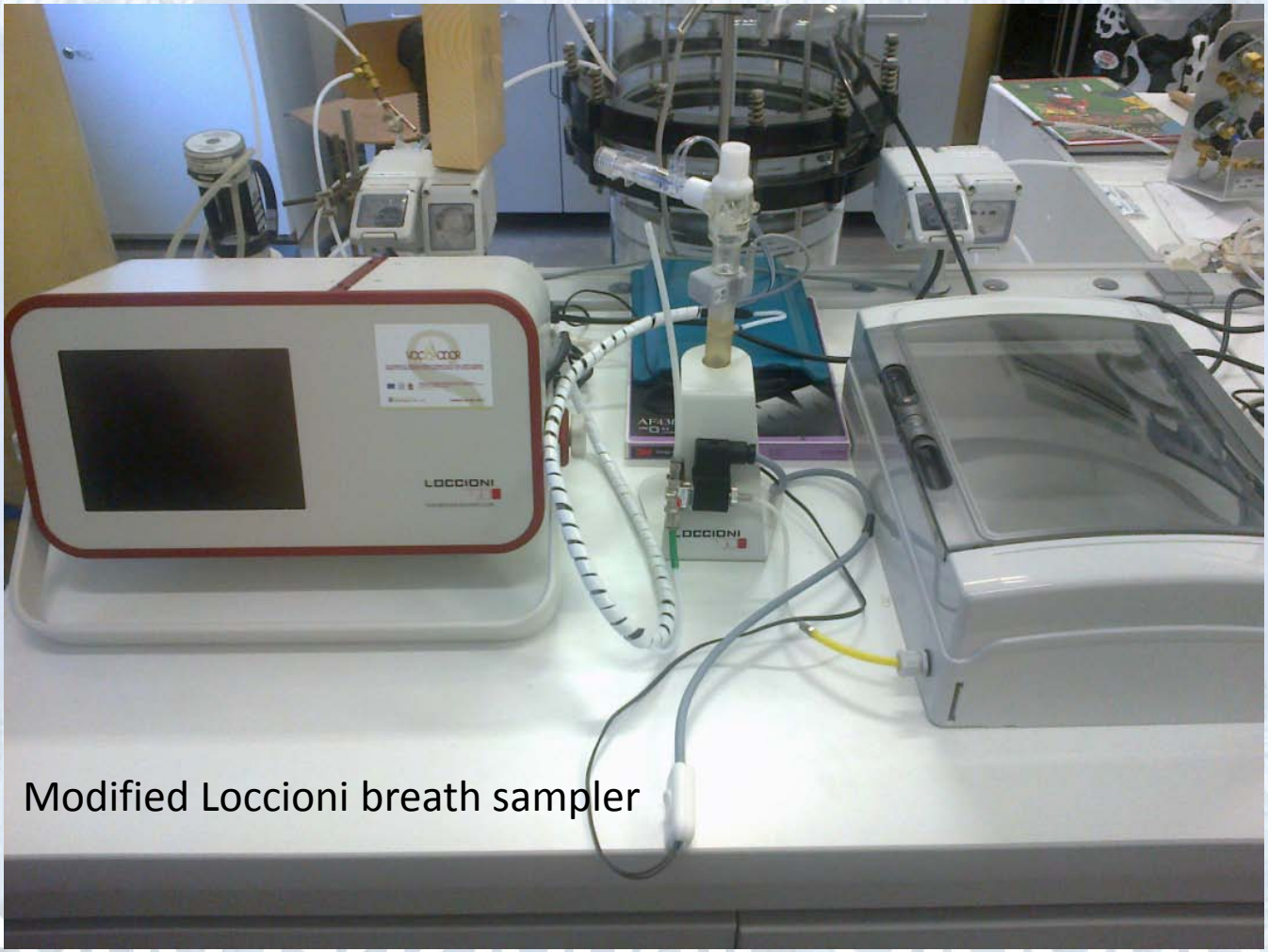
Silice



One way valve

Collecting bag (in Tedlar)

selected alveolar breath sampling



Modified Loccioni breath sampler

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VOCs with higher discriminant value in the breath of CRC patients

11 VOCs
1,2-pentadiene
2-methylbutane
3-methylpentane
methylcyclopentane
cyclohexane
decanal
nonanal
methylcyclohexane
4-methyl-2-pentanone
1,4-dimethylbenzene
1,3-dimethylbenzene

Original article **Br. J Surg. 2013**

Exhaled volatile organic compounds identify patients with colorectal cancer

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Correspondence to: Professor D. F. Altomare, Department of Emergency and Organ Transplantation, c/o Azienda Ospedaliero-Universitaria Policlinico Bari, Piazza G. Cesare, 11-70124 Bari, Italy (e-mail: donatofrancesco.altomare@uniba.it)

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Annals of Surgery Volume 262, Number 5, November 2015

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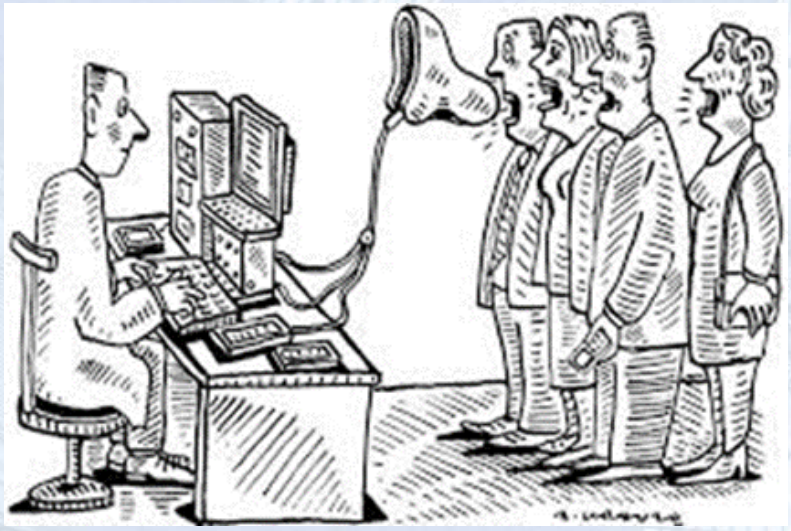
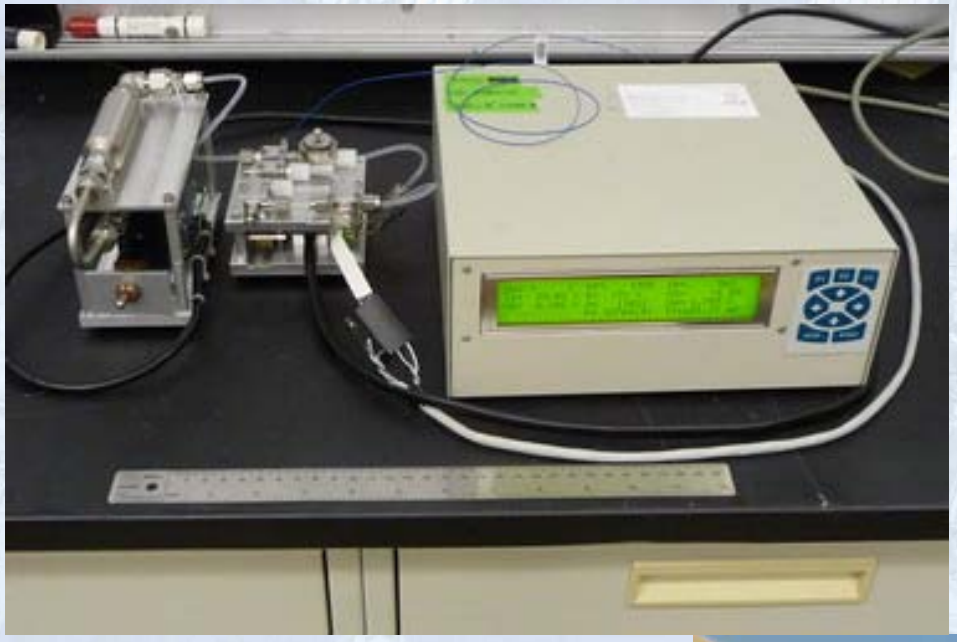
- Identification of the pattern of VOCs involved
  - Testing the specificity of the VOCs pattern for this diseases compared to intestinal polyps, IBD
  - Testing the reliability of the test compared to FIT
- **Availability of an easy usable and portable device for online analysis**
  - E nose
  - QEPAS
  - Odour-binding Proteins

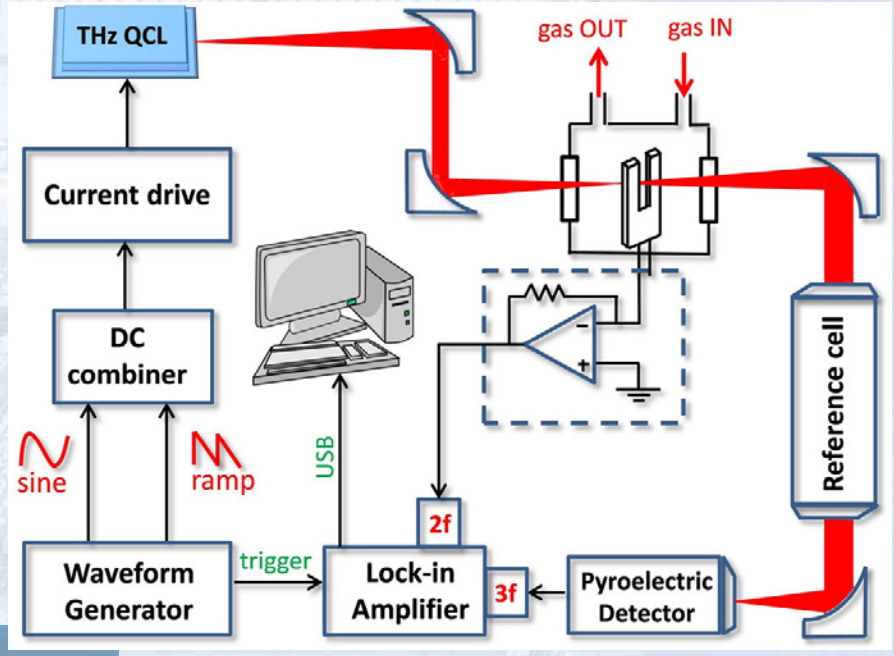
VOCs and human cancer
…electronic nose for the future…..



«In the not too distant future blowing into a breath-analyser will become as routine as having a blood test»

Quartz-Enhanced Photo-Acoustic Sensors (QEPAS) for the sensitive and selective quantification of molecular trace gas species





VOCs and human cancer

…the diagnosis is in the air……

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Population

- 48 CRC pts
- M/F=1.4
- mean age 63 y
- submitted to curative resection
- stage I-II: 28 stage III-IV: 20

- 32/48 pts disease free
- M/F=1.7
- mean age 62 y
- Mean follow-up of 24 months
- Original stage:

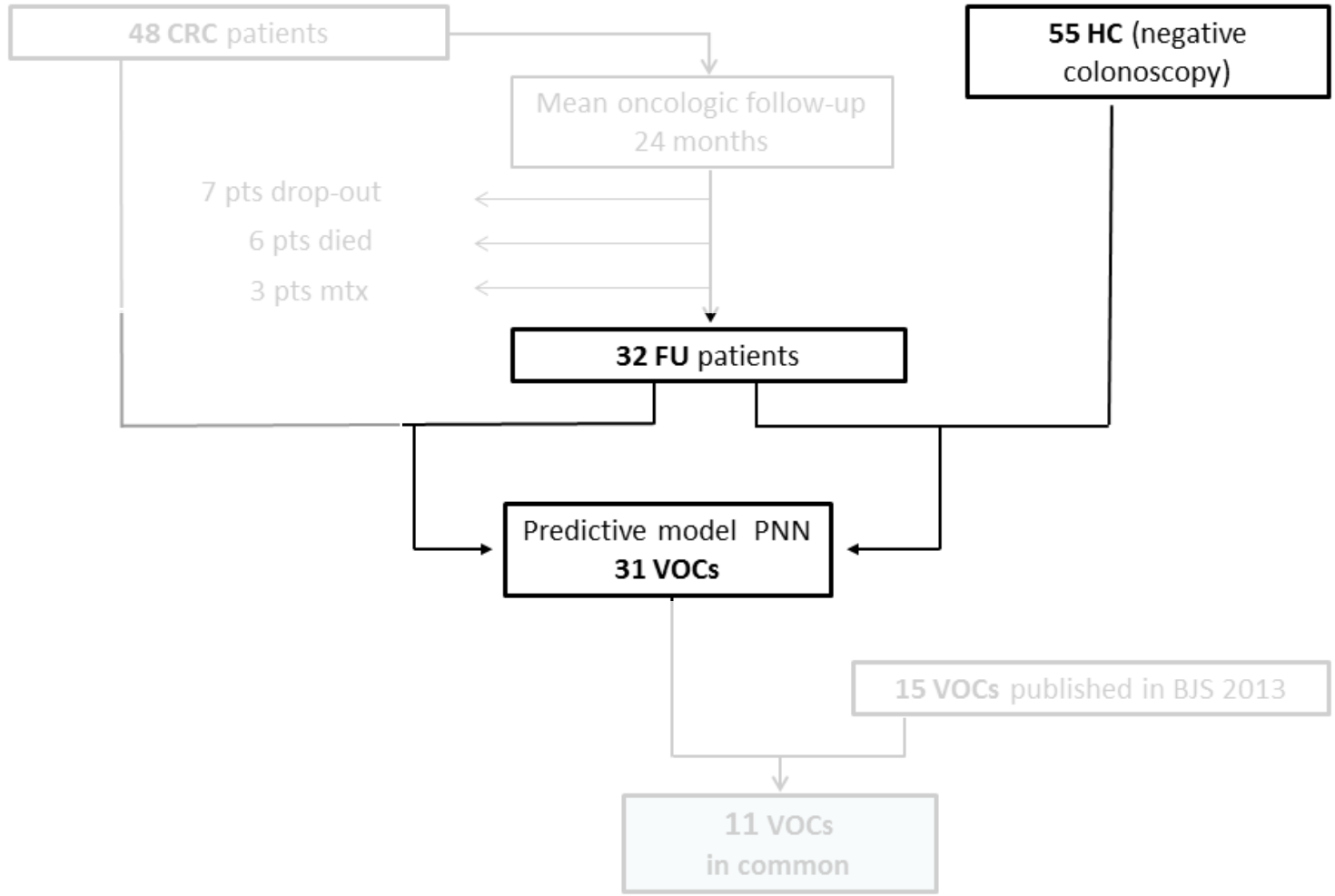
  I-II: 20 pts

  III-IV: 12 pts

- 55 healthy controls
- M/F=1.03
- mean age 49.5y
- negative colonoscopy for screening purpose

Design of the study 2.

VOCs analysis in patients disease free in follow-up and healthy controls



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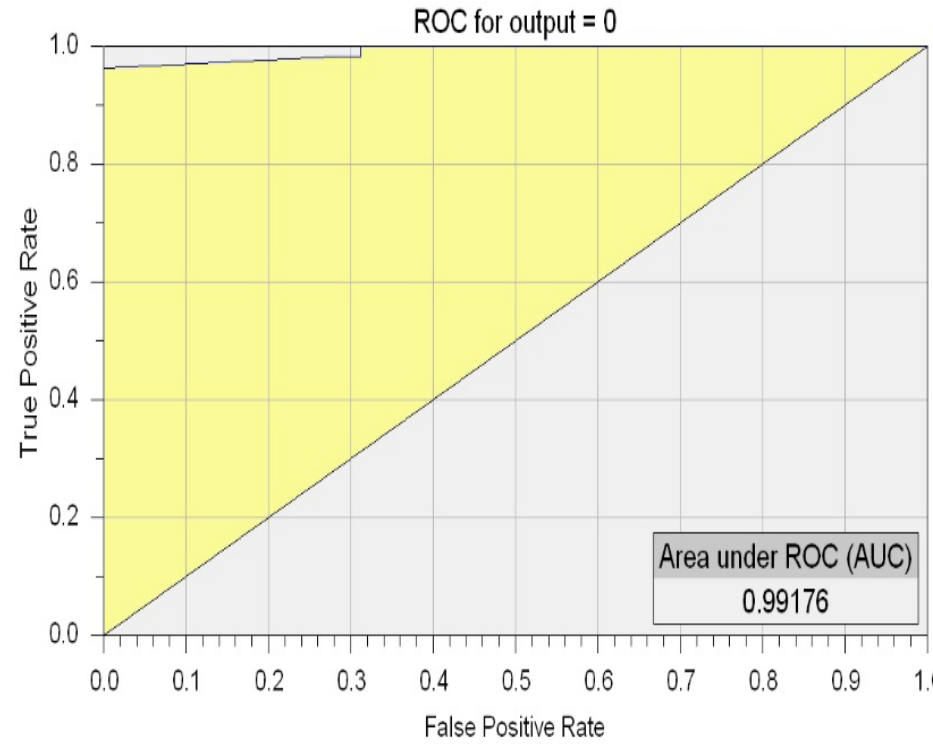
methylcyclopentane	undecane
cyclohexane	Y*
heptane	nonanal
methylcyclohexane	dodecane
4-methyl-2-pentanone	decanal
1-methylnaphthalene	1-ethyl-1,2,4-trimethylbenzene
1-octene	1-ethyl-2,4,5-trimethylbenzene
octane	2,3-dihydro-1,6-dimethyl-1H-Indene
1,2,3-trimethylbenzene	2,3-dihydro-4,7-dimethyl-1H-Indene
1,3-dimethylbenzene	1,3-dimethyl-5-(1-methylethyl)benzene
1,4-dimethylbenzene	2-methylnaphthalene
propylbenzene	

Disease free patients in follow-up vs healthy controls

PNN analysis

SENSITIVITY	**100 %**
SPECIFICITY	**96.36%**
ACCURACY	97.70 %
AUC	0.992



VOCs identified in common with our previously study *(Br J Surg 2013)*

11 VOCs
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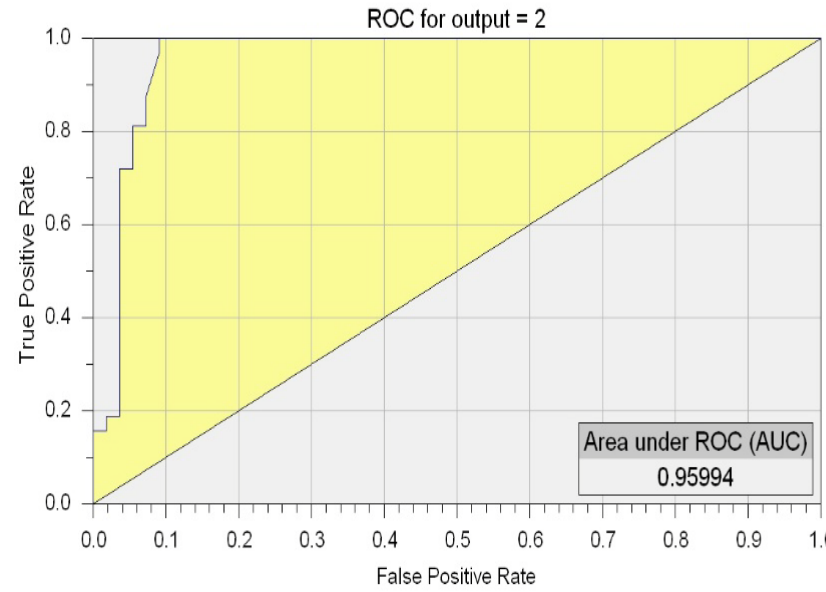
nonanal
methylcyclohexane
4-methyl-2-pentanone
1,4-dimethylbenzene
1,3-dimethylbenzene

Disease free patients in follow-up vs healthy controls

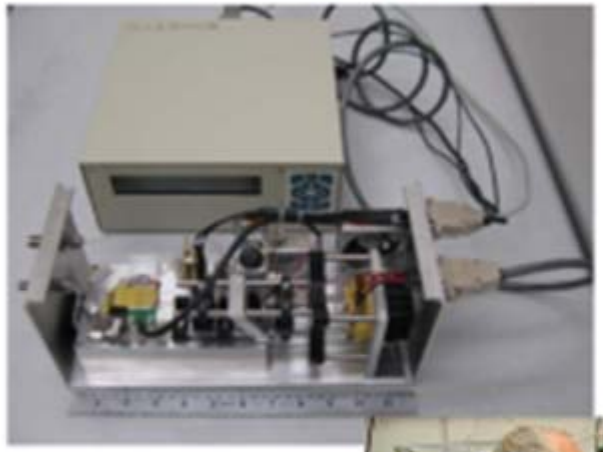
SENSITIVITY	**100 %**
SPECIFICITY	**90.91%**
ACCURACY	94.25%
AUC	0.959

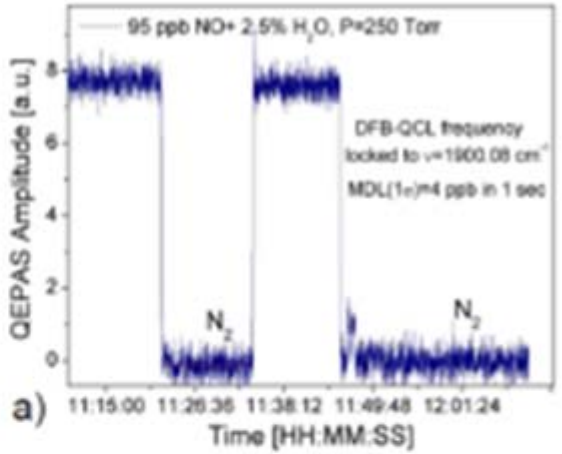


Development of quartz-enhanced photoacoustic sensors (QEPAS) for the sensitive and selective quantification of molecular trace gas species with resolved spectroscopic features

QEPAS Breath Sensors

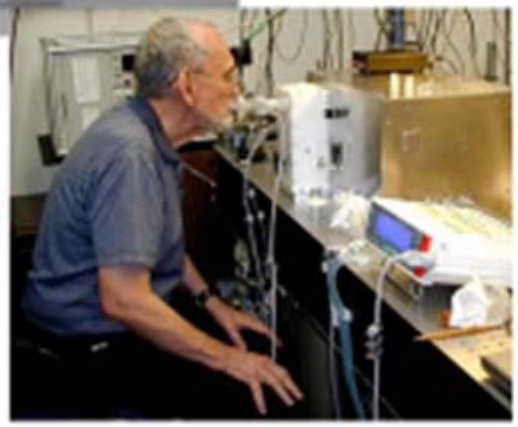
NO Breath Sensor

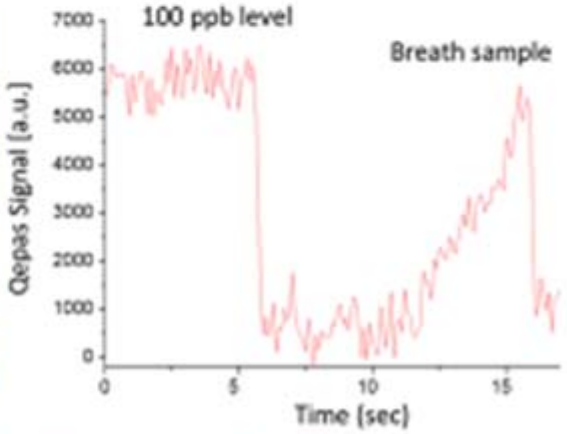




Collaborazione con Rice Univ.

RICE





Test Clinici nel Houston Medical Center in Texas

Performance of CRC screening tests

	FOBT	FIT	Colonography	Colonoscopy
Participation rate (%)	**16-47**	**11-77**	**18-34**	**16-93**
Positive rate (%)	**2.4-6.8**	**1.1-1.3**	**8.6-9**	**4.9-11**
Detected adv cancer/1000 invited individuals	**2.1-6.3**	**1.1-21**	**8.8-21**	**14-73**