Respiratory Physiology & Neurobiology 187 (2013) 128–134

Contents lists available at SciVerse ScienceDirect

Respiratory Physiology & Neurobiology

journal homepage: www.elsevier.com/locate/resphysiol

Review

Pathologies currently identified by exhaled biomarkers☆

Andrea Mazzatenta[a,b], Camillo Di Giulio[a], Mieczyslaw Pokorski[c,d,*]

[a] Unit of Sensory Physiology, Department of Neuroscience and Imaging, University of Chieti-Pescara 'G. d'Annunzio', Italy
[b] Neuroscience Institute, National Center of Research, Pisa, Italy
[c] Department of Respiratory Research, Medical Research Center, Polish Academy of Sciences, Warsaw, Poland
[d] Institute of Psychology, Opole University, Opole, Poland

ARTICLE INFO

Article history:
Accepted 19 February 2013

Keywords:
Brain diseases
Chronic fatigue
Cognition
Exhaled breath
Oxidative stress
Volatile organic compounds (VOCs)

ABSTRACT

Ancient Greek physicians already knew that the smell of human breath could provide a clue to the pathology. Nowadays, volatile breath biomarkers are known to be released in a broad range of diseases. However, their identification, isolation, and quantification as indicative of relevant alterations in clinical status have required the development of new techniques and analytical methods. Breath sample analysis encounters several obstacles. Particularly, there is a need of a system that could work in a continuous manner, with the low concentration and small volume of a sample. Herein we review, in the light of literature and our experience, clinical applications of the metal oxide semiconductor (MOS) sensor for breath analysis to distinguish between health and disease in some conditions, e.g., diabetes, multiple chemical sensitivity (MCS) syndrome, or in tracing the central neural fatigue resulting from cognitive performance. We submit that exhaled breath analysis holds promise in the diagnosis and treatment of genetic or neurodegenerative diseases which involve cognitive derangements.

1. Introduction

Biophysical, biochemical, and molecular biological methods have mainly been developed for blood and urine analysis in medical monitoring equipments and diagnostics. Comparatively, diagnostics based on breath analysis, which are among the least invasive methods for monitoring person's health, are less developed and not yet regularly used in clinical practice. The applications of breath tests are valuable in diagnosis of disease, including aging and neurodegenerative pathology, and in the assessment of exposure to environmental pollutants or drugs (Risby and Sehnert, 1999; Cao and Duan, 2006).

Gas exchange at the alveolar-blood capillary membrane of the respiratory tract is essential to life. This mechanism is a passive diffusion driven by the carbon dioxide and oxygen unbalanced concentration gradients. Following these vital gasses, molecules present either in the blood or in inhaled air can also diffuse passively into the breath or blood, respectively. Interestingly, exhaled breath could be characterized by a distinctive smell. These unique stenches have been used, since the time of Hippocrates, as indicators of several diseases: diabetes, lung, liver or renal pathology, sepsis, or periodontal infections (Phillips, 1992). These intuitive observations have later been proven by using classical analytical methods. The molecular profile of breath has been characterized in concentrations and identities of the compounds in healthy and pathological conditions (for review see Miekisch et al., 2004). Principal components up to 99% are few compounds: nitrogen, oxygen, carbon dioxide, water vapor, and the inert gases. The residue consists of a mixture of many molecules with concentrations in the range of parts per million (ppm) to parts per trillion (ppt) by volume (Chen et al., 1970; Pauling et al., 1971; Riely et al., 1974; Dannecker et al., 1981; Solga and Risby, 2010). In normal subjects, more than 3400 different volatile organic compounds (VOCs) can be detected in the exhaled breath; however, only a small fraction of these VOCs are present in all subjects. These are principally isoprene, alkanes, methylalkane, and benzene derivatives (Phillips et al., 1999). Intuitively, this residual part is the most interesting for searching biomarkers of pathological conditions (Risby, 2002). However, given the minute concentration of these molecules, it is essential to investigate breath by the application of new generation of analytical instruments capable of high resolution detection (Risby and Solga, 2006; Solga and Risby, 2010). This review is intended to describe the broad range of applications of breath analysis, including a new sensor generation and its potential for clinical diagnosis of new diseases and a real time monitoring during cognitive performance tests.

☆ This paper is part of a special issue entitled "Immunopathology of the Respiratory System", guest-edited by Professor Mieczyslaw Pokorski.

* Corresponding author at: Department of Respiratory Research, Medical Research Center, Polish Academy of Sciences, 5 Pawinskiego St., 02-106 Warsaw, Poland. Tel.: +48 22 6086622.
E-mail addresses: m.pokorski@hotmail.com, mieczyslaw.pokorski@gmail.com (M. Pokorski).

1569-9048/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.resp.2013.02.016

2. Breath analysis techniques, limitations and future perspectives

Capnography is a classic monitoring system of the concentration or partial pressure of carbon dioxide (CO_2) in the respiratory gases. It provides information about CO_2 production, lung perfusion, alveolar ventilation, respiratory patterns, indirect metabolism measurement, and elimination of CO_2 after anesthesia from the breathing circuit and ventilator. It is usually represented as a graph, the capnogram, of expiratory CO_2 plotted against time or expired volume. The capnogram is a direct monitor of the inhaled and exhaled CO_2, and an indirect monitor of its partial pressure in the arterial blood. The capnogram cycle is composed by four phases. In summary, the initial exhalation consists of predominantly anatomical dead space air (~21% O_2, 0.03% CO_2, 78% N_2, and 0.5% H_2O), and is followed by alveolar gas and a rapid rise in CO_2 which reaches a clear plateau in normal lungs and is termed the end-tidal concentration, which corresponds to the concentration of carbon dioxide in venous blood; the composition of breath at the end of the third phase is about 13% O_2, 5% CO_2, 78% N_2, and 4% H_2O; the last phase is the start of inhalation for the subsequent breath. The shape of the capnogram curve is affected by obstructive conditions such as bronchitis, emphysema and asthma, or chronic obstructive pulmonary disease (COPD). The capnography limitations are: composition and concentration of a breath sample will vary significantly over the breathing cycle; breathing velocity will affect the rate of mixing between alveolar gas and dead space air; the depth and frequency of breathing are under autonomic control, and when the subject is asked to provide a breath sample, this action invariably results in his changing from autonomic to conscious breathing (Cope et al., 2004; Amann et al., 2010).

In early studies devoted to breath biomarkers, standard indirect analytical chemistry techniques were used, typically consisting of gas chromatography (GC) (Jansson and Larsson, 1969; Chen et al., 1970; Pauling et al., 1971; Riely et al., 1974; Dannecker et al., 1981). This technique allowed an indirect identification of molecules present in breath at concentrations greater than 40 μmol/ml, which is its major limitation. In order to increase the sensitivity of the technique, several methods of breath sample concentration were adopted: cryogenic trapping and adsorption onto carbonaceous surfaces or hydrophobic polymeric sorbents. However, even with a concentration method only a small portion, less than a 100 ml of the total volume of an individual breath, which is typically about 4.7 l/min, can be analyzed. Furthermore, it is debatable if an individual breath sampled is representative of all previous and subsequent breaths. The sampling of a representative breath(s) in controlled conditions is an essential requirement for breath analysis research (Risby and Solga, 2006). Moreover, to identify a broad range of VOCs markers in alveolar exhale, the breath methylated alkane contour (BMAC) was used. The BMAC incorporates a comprehensive spectrum of markers of oxidative stress. The assay analyzed VOCs by gas chromatography coupled with mass spectroscopy to construct the BMAC, a 3-dimensional display of the abundance of C_4 to C_{20} alkanes and monomethylated alkanes as a function of carbon chain length. A breath collector apparatus captures VOCs usually in 1.0 l breath and in 1.0 l room air onto separate sorbent traps. VOCs in the sorbent traps were analyzed by automated thermal desorption, gas chromatography, and mass spectroscopy (Phillips, 1997).

Exhaled breath condensate is collected by breathing directly through a cooling device, resulting in the accumulation of exhaled breath constituents in the cooling chamber. The principal component of exhaled breath condensate is water vapor, representing nearly all of the volume (99%) of a sample. Only is a small fraction of the condensate derived from respiratory droplets containing nonvolatile molecules. Exhaled breath condensate contains large number of ions, metabolites, and other molecules, including adenosine, ATP, ammonia, hydrogen peroxide, isoprostanes, lactate, leukotrienes, nitrogen oxides, peptides, prostaglandins, tromboxanes, and various cytokines (Horvath et al., 2005). The concentration of different mediators is influenced by lung diseases and modulated by therapeutic interventions.

The measurement of fractional exhaled nitric oxide (FeNO) is a quantitative measure of airway NO, a gaseous mediator produced endogenously in cells under the stimulus of cytokines by NO synthases (Dweik et al., 2011; Szefler et al., 2012). FeNO analysis is successfully employed in several disorders, mainly asthma. Direct breath analysis is currently receiving significant attention since this approach does not involve sample loss due to the irreversible adsorption onto the surface of sampling media and enables to quantify reactive breath molecules. Direct analytical methods are based upon electrochemistry, chemical sensors, optical spectroscopy, mass spectroscopy (MS, selected ion flow tube mass spectroscopy (SIFT-MS), ion mobility spectroscopy (IMS), differential mobility spectroscopy (DMS), proton transfer mass spectroscopy (PTR-MS), or fast gas chromatography (for review see Amann et al., 2010). A disadvantage of direct sampling is that detection limits cannot be enhanced by pre-concentration of the breath.

The application of the electronic nose (EN) in breath analysis was an extension of the direct methods for the smellprint identification. ENs rely on arrays of chemical vapor sensors that respond to specific characteristics of an odorant molecule, including VOCs. The EN, like the human olfactory system, generates a smellprint that can be compared with previously stored ones. The ENs has been successful in discriminating between the smellprints of healthy subjects and patients with asthma, lung cancer, and COPD sufferers (Montuschi et al., 2000; Di Natale et al., 2003; Machado et al., 2005). Clinical application of ENs for detection of lung cancer offers several potential advantages, but it has disadvantages as well. The advantages include high sensitivity, ease of administration of the test and portability of the detector. The disadvantages include loss of sensitivity in the presence of water vapors or high concentrations of a single component, sensor drift, and the inability to provide absolute calibration, relatively short life of some sensors, necessity to do considerable method development work for each specific application, and the inability to obtain quantitative data (Horvath et al., 2009). Another analytical system based on colorimetric sensor array with 36 spots composed of different chemically sensitive compounds impregnated on a disposable cartridge has been used to detect lung cancer from exhaled breath. The color of the spots changes under the influence of chemicals with which they come into contact (Mazzone et al., 2007).

Interestingly, smellprint of diseases, at least cancer, is also recognized by dogs. By the virtue of sensitivity of their smelling and their capacity to learn how to distinguish differences, dogs can be trained to discriminate exhaled breath samples from subjects with and without lung cancer. In a double-blind study, dogs had an accuracy of 99% in discriminating between smells from exhaled breath of patients with lung cancer and controls. The stage of cancer, age of patients, smoking habit, or most recently eaten meal did not influence the dog's diagnostic performance (Williams and Pembroke, 1989).

Laser optic methods, arrays of nano- and mesowire sensors, and bioelectronic noses based on mammalian olfactory receptors in immobilized nanosomes represent a new generation of electronic noses for detection and discrimination of volatiles, which may also be used in breath research (Mazzone et al., 2007; Spanel and Smith, 2008; Skeldon et al., 2006; Thorpe et al., 2008; Sysoev et al., 2006; Vidic et al., 2006; Radhika et al., 2007). A new generation of sensor metal oxide semiconductor (MOS) is suitable for real time monitoring. In particular, the real-time breath analysis is applicable for both diagnosis and during performing cognitive tasks (Mazzatenta et al., 2013a,b). MOS sensor is able to detect a

broad range of volatile compounds (both organic and inorganic, e.g., alcohols, aldehydes, aliphatic hydrocarbons, amines, aromatic hydrocarbons, ketones, organic acids, and CO), while correlating directly with the CO_2 level. The MOS sensor is based on a chemical reaction which occurs between the surface of the sensor and the volatile compound. Its limitation lies in the discrimination of different compounds. In the next section, clinical application of these methods indirect or direct, or real time will be described in cross-sectional and longitudinal studies.

3. Clinical application of breath analysis

Breath analysis has been applied both in cross-sectional and longitudinal studies (Miekisch et al., 2004). In cross-sectional studies, a control group is compared with a disease group, and breath biomarkers are analyzed to identify qualitative or quantitative differences between the two groups. In longitudinal studies, breath biomarkers are observed during the course of a disease or monitoring of pharmacologic interventions. As a result of these studies, several breath biomarkers have been identified and used in diagnosis of disease (Risby and Solga, 2006; Solga and Risby, 2010).

3.1. Aging, inflammation, and oxidative stress

The aging process is based upon the adverse effects of oxidative stress, which increase with advancing age (Ashok and Ali, 1999). Oxidative stress is a condition in which cells are damaged as a result of a chemical reaction with oxidative agents, such as the superoxide anion (O_2^-) or hydroxyl radical (OH^-). These reactive oxygen species (ROS), however, originate both in senescence, leak from mitochondria into the cytoplasm; and in inflammation process, generated by activated granulocytes that act physiologically as defense mechanisms against microbial attack (Metidiewa and Koska, 2000). ROS cause cellular damage by oxidizing a variety of biologically important molecules, proteins, lipids and DNA, accelerates shortening of telomeres and the mitotic clock in human somatic cells, until eventually a critical deletion causes cellular senescence and death (Ashok and Ali, 1999). Clinical studies have demonstrated a close correlation between pathological status with high inflammation and the exhalation of hydrocarbons generated through ROS attack on membrane lipid structures. Ethane and pentane are generated in this way from ω-3 and ω-6 fatty acids, which are basic components of cell membranes, and also other lipid peroxidation markers such as aldehydes malondialdehyde (MDA), thiobarbituric acid-reactive substances, and glutathione (Aghdassi and Allard, 2000; Dumelin and Tappel, 1977). Interestingly, reduced levels of the antioxidants selenium and vitamin E synergistically promote tissue polyunsaturated fatty acids peroxidation, generating alkanes and elevated levels of breath ethane. Oxidative stress is among the most frequent pathologic conditions in critical illness. Multiorgan dysfunction or failure, which is among the leading causes of morbidity and mortality in critical care, is a result of oxidative stress. Oxidative status in humans can be measured through urine, plasma, blood, and breath. Breath markers are often more sensitive than the serum markers MDA and thiobarbituric acid-reactive substances (Phillips et al., 2004; Risby and Sehnert, 1999). Hydrocarbons, as stable end-products of lipid peroxidation, show low solubility in blood and are excreted into the breath within minutes of their formation in tissues. Exhaled concentrations of alkanes can therefore be used to monitor the degree of oxidative damage (Aghdassi and Allard, 2000). In addition, another breath marker of oxidative stress in lung diseases as well as a marker of oxygen radical-mediated tissue damage in cystic fibrosis is H_2O_2 measured in breath condensate (Lases et al., 2000). H_2O_2 is formed by inflammatory cells in the upper and lower airways. In general, H_2O_2 is detected in the exhaled breath condensate.

3.2. Lung diseases

In lung diseases such as asthma, chronic obstructive pulmonary disease (COPD), bronchiectasis, cystic fibrosis (CF), interstitial lung disease, obstructive sleep apnea syndrome (OSAS), adult respiratory distress syndrome (ARDS), pulmonary allograft dysfunction, lung cancer characteristically chronic inflammation and oxidative stress are involved (Cao and Duan, 2006). As one of the most important breath marker of lung diseases, exhaled nitric oxide (NO) has extensively been studied.

Asthma is a chronic inflammatory disorder of the airways that produces airway hyperresponsiveness, reversible airway obstruction, and symptoms such as wheezing, cough, and shortness of breath. Asthma sufferers have been shown to exhale high levels of NO, which is generally accepted as an indirect marker of airway inflammation and is a supplemental biomarker in asthma (Rosias et al., 2004). In fact, an increase in exhaled NO is not specific for asthma, but an increased concentration may be useful in differentiating asthma from other causes of chronic cough. NO is synthesized by 3 isoforms of nitric oxide synthase (NOS) encoded by 3 distinct genes localized on chromosomes 7, 12, and 17: NOS1, NOS2A, and NOS3. This molecule is released into the circulation, where it acts as both vasodilator and chemotactic agent for neutrophils, and in airways as exhaled gas. In general, the upper value of normal, for online measure, of FeNO is 25 ppb (Dweik et al., 2011; Szefler et al., 2012). In patients with stable well-controlled asthma, FeNO values have been reported to range from 22 to 44 ppb (Olin et al., 2006). A clinically important decrease of FeNO is defined as a change of 20% for values over 50 ppb (or a change of 10 ppb for values lower than 50 ppb) that occurs 2–6 weeks after initiation of corticosteroid therapy (Szefler et al., 2002; Dweik et al., 2011). FeNO measured at a constant flow rate is a simple, safe, and reproducible biomarker for use in asthma clinical trials. In online measurement, the subject exhales directly into the instruments, while in offline measurement exhaled air is collected in a specific bag for later NO estimation. The measurement methods are well described in the American Thoracic Society (ATS) and European Respiratory Society (ERS) guidelines (2005). FeNO is recommended as a supplemental outcome in clinical trials that seek to evaluate the effects of interventions in asthma and to characterize its corticosteroid-responsive phenotypes (Szefler et al., 2012). Interestingly, the application of the electronic nose was successful in discrimination between healthy subjects and patients with asthma, and between patients with asthma of different severity, besides patients with lung cancer, and between these and COPD sufferers (Montuschi et al., 2000). H_2O_2 is another biomarker that appears to increase in the exhaled breath condensate in patients with asthma and is related to the total number of eosinophils.

In obstructive sleep apnea syndrome (OSAS) airway inflammation and oxidative stress are involved. In particular, obesity increases systemic and pulmonary inflammation. Although the relation between obesity and systemic inflammation is known, the link between obesity and pulmonary inflammation still remains unclear. Certainly adipose tissue is an important source of cytokines, collectively called adipokines, which are implicated in systemic inflammation, and probably these adipokines could play a fundamental role in increasing pulmonary inflammation, too. NO has been found to be increased in OSAS, even though the mechanisms involved in NO production in OSAS remain complex. However, several other biomarkers are found in exhaled breath condensate of OSAS patients, together with pentane and CO (Carpagnano, 2011).

COPD is characterized by progressive airflow obstruction that is not fully reversible and correlate with chronic inflammation in response to noxious particles or gases (e.g., cigarette smoking).

According to the WHO Global Burden of Disease Study, COPD is the fifth leading cause of death worldwide (Mathers and Loncar, 2006). COPD is a complex disease with multiple pathological components, the best known inherited risk factor for early-onset COPD is genetically deficient plasma levels of α1-antitrypsin (Dahl and Nordestgaard, 2009). FeNO is related to the severity of COPD and it may be a useful biomarker (Clini et al., 1998). Furthermore, exhaled breath condensate analysis in patients with COPD encompasses other related breath markers, including H_2O_2, eicosanoids (leukotrienes, prostanoids, and isoprostanes), NO-derived products (S-nitrosothiols, nitrite and nitrate), pH, or aldehydes (Montuschi, 2005).

Lung transplantation has now become acceptable palliation for the end-stage consequences of many pulmonary diseases: COPD, idiopathic pulmonary fibrosis (IPF), cystic fibrosis (CF), and α1-antitrypsin deficiency emphysema (AAT) (Christie et al., 2008). Chronic rejection has been dubbed the 'Achilles heel' of long-term lung transplant success (Orens and Garrity, 2009). Chronic transplant deterioration occurs as a consequence of fibrous obliteration of small airways. This is known as the bronchiolitis obliterans syndrome (BOS), which is a persistent drop in forced expiratory volume in one second (FEV1) by 20% or more in the absence of other identifiable causes. Furthermore, the incidence of the gastroesophageal reflux (GER) increases in the lung transplant recipients (Hadjiliadis et al., 2003). Exhaled breath condensate analysis, coupled to esophageal manometry and standard esophageal pH monitoring to identifying reflux, offers a unique potential in the aspiration workup, with the added benefit of safety in the lung transplant recipients. The condensate can then be collected and assayed for numerous biomarkers of disease, notably protons (by means of pH), hydrogen peroxide, nitric oxide, and cytokines (Fisher et al., 1998; Hunt, 2002).

Lung cancer is one of the leading cause of cancer death today. Several approaches, ranging from breath methylated alkane contour (BMAC) to electronic nose (EN), were used to investigate breath in lung cancer patients and to identify cancer biomarkers or smellprint. For instance, in alveolar gradients (i.e., the abundance in breath minus that in room air) of C_4 to C_{20} alkanes and monomethylated alkanes in the breath have provided a rational new set of markers in primary lung cancer (Phillips et al., 2003). The EN, based on quartz microbalance sensors covered by metalloporphyrins to bind different VOCs, identified a smellprint characteristic of lung cancer, showing a 90% accuracy (Di Natale et al., 2003). Another type of EN, comprising of 32 separate carbon polymer sensors, found a difference between the smellprints of lung cancer patients and that of controls, which included both healthy nonsmokers and also patients with different lung diseases. However, no signal difference was observed between patients with different clinical severity of the disease and no significant confounding effect of cigarette smoking and accompanying respiratory diseases, such as asthma and COPD, were detected (Machado et al., 2005). In an in vitro study, tumor and normal cell lines were suspended in saline and a polymer composite electronic nose was used to evaluate the headspace gases. In the tumor cell lines, including adenocarcinoma, squamous cell carcinoma, and mesothelioma, smellprints were distinct from each other and from the normal fibroblast and smooth muscle cells (Gendron et al., 2007).

Dogs, trained to sniff cancer, provide further evidence that metabolic pathways of tumor cells produce different volatile compounds (or at least a different pattern of them) than in noncancerous tissue. VOCs produced by tumors and detected by dogs are the products of major histocompatibility complex (MHC) genes (Balseiro and Correia, 2006). These human leukocyte antigen (HLA) molecules have soluble isoforms that are present in blood, urine and sweat, and MHC-dependent odor components can be detected by an electronic nose (Montag et al., 2001).

3.3. Metabolic disorder

Metabolism maintains homeostasis. A metabolic disorder is any problem in the body that causes loss of body homeostasis. Metabolic disorders include diabetes, phenylketonuria, metabolic syndrome, sodium metabolism disorders, calcium metabolism disorders, hyper- and hypocalcemia, potassium metabolism disorders, hyper- and hypokalemia, phosphate metabolism disorders, magnesium metabolism disorders, or acid–base metabolism disorders. Type 2 diabetes is an archetype metabolic disorder that is characterized by high blood glucose in the context of insulin resistance and relative insulin deficiency; it makes up about 90% of cases of diabetes. Diabetes mellitus type 1, in which there is an absolute insulin deficiency due to destruction of islet cells in the pancreas, along with the gestational diabetes, constitutes the remaining 10%. Diabetes is a large and growing problem throughout the world's developed and developing nations. It is characterized by increases in glucose concentrations and intensive lipolysis. These metabolic alterations induce production of ketone bodies by the liver and used peripherally as a substitute of the glucose as energy source. Ketone bodies consist of acetoacetate, 3-β-hydroxybutyrate, and acetone. The gas-phase acetone in the blood equilibrates with alveolar air through the alveoli. Therefore, the concentration of acetone in breath can reflect metabolic products of diabetes. In healthy individuals, breath acetone concentrations are in the range of a few hundred ppb (by volume) (Henderson et al., 1952). Persons with diabetes have a much broader range of breath acetone concentrations, which can be as high as 560 ppm or even >1000 ppm depending on the characteristics of an individual (e.g., age, sex, and ethnic group) and on the blood glucose concentration. Breath acetone extensively correlates with blood glucose and is a useful candidate breath marker in diabetes (Crofford et al., 1977; Smith et al., 1999).

Breath hydrogen measurements have been applied in clinical medicine for the detection of carbohydrate malabsorption. H_2 is produced by bacterial metabolism of carbohydrates when dietary sugars escape absorption in the small intestine, it is absorbed into the portal circulation and excreted in the breath. H_2 breath tests can be used to diagnose clinical disorders of digestion and absorption, including lactase deficiency and other disorders of di- and monosaccharide or starch malabsorption, and small-bowel bacterial overgrowth (Perman, 1991).

The urea breath test (UBT) is one of the most successful breath tests used in clinical applications. The range of diseases that can be identified include *Helicobacter pylori* infection, lactose and fructose intolerance, bacterial overgrowth, bile salt wastage, pancreatic insufficiency, liver dysfunction, and abnormal small-bowel transit (Romagnuolo et al., 2002). Urea usually is produced by the body from excess ('waste') nitrogen and then eliminated in the urine. For the UBT, patients swallow a capsule containing urea labeled with ^{13}C or ^{14}C. If *H. pylori* is present in the stomach, the bacterium metabolizes the urea into nitrogen and carbon (as CO_2). The CO_2 is absorbed across the lining of the stomach and into the blood. It is then excreted from the lungs in the breath.

In hepatic disorders, increased levels of exhaled breath hydrocarbons, such as ethane, have been utilized to investigate the generation of free radicals during dialysis for kidney failure (Risby and Sehnert, 1999)

3.4. Environmental diseases, multiple chemical sensitivity (MCS)

MCS is a complex disorder initiated by chemical exposure, particularly through the airways. MCS patients report sensitivity or intolerance to low levels of a wide spectrum of chemicals. Symptoms could include asthma-like signs, rhinitis, fatigue, cognitive dysfunction, psycho-physiological alteration, and other specific

tissue reactions resembling hypoxic and oxidative stress effects. To recognize physiological signs that would allow the diagnosis of MCS in a non-invasive way, we investigated the potential application of a new sensor system (Mazzatenta et al., 2013a). In healthy volunteers, we measured exhaled breath content in the control condition and under exposure to olfactory stressors (n-propanol) that mimic hypoxic or pollutant stressors playing a role in the MCS development. The recording system used is based on a metal oxide semiconductor (MOS) sensor having a sensing range of 450–2000 ppm CO_2 equivalents, which is able to detect a broad range of compounds that may underlie the MCS, while correlating directly with the CO_2 levels. The results indicate that the recording system employed was suitable for the analysis of VOCs exhaled breath content and breath parameters in humans. Interestingly, the system was able to detect and discriminate between the exhaled breath VOCs content taken from the control condition and those from conditions under stress that mimicked exposures to pollutant or hypoxia. The results suggest that chronic hypoxia and oxidative stress could be involved in the MCS disorder.

4. Future perspective in breath analysis

Clinical usefulness of breath analysis has been well demonstrated in several diagnostic applications; however, a new future in breath analysis is upcoming at an accelerating pace. We have investigated and quantified exhaled VOCs while performing cognitive tasks, consisting of solving the Sudoku puzzles, in patients suffering from diabetes type 2 and in control subjects, using a MOS sensor. The rationale for that was that the brain target areas for insulin are particularly located in the hypothalamus and hippocampus, both involved in cognition and body homeostasis regulation. We found not only basic differences in the basal level of VOCs emissions between the diabetic and control subjects, but also interestingly while taxing the brain with a cognitive effort

Table 1
Typical concentration of molecules found in human breath and physiological origin (modified from Risby).

Compound	Concentr. (v/v)	Physiological basis	Clinical applications
Acetaldehyde	Ppb	Ethanol metabolism	Hepatic function
Acetone	Ppb	Fatty acids metabolism	Diabetes
Alkylamines	Ppb	–	Renal function
Ammonia	Ppb	Protein metabolism	Renal function, hepatic function, urea cycle disorder, hepatic encephalitis, exercise
2-Aminoacetophenone	Ppb	Metabolic products of bacteria	Infections
Carbon dioxide	%	Respiration	CO_2 production, lung perfusion, alveolar ventilation, respiratory patterns, indirect metabolism measurement, CO_2 elimination after anesthesia and from ventilators
Carbon monoxide	Ppm	Heme catabolism catalyzed by heme oxygenase cytoprotective role	Host response to infection, induction of antioxidant defenses
Carbonyl sulfide	Ppb	Gut bacterial oxidation of reduced sulfur species	Lung transplant recipient with acute rejection, hepatic function
Ethane	Ppb	Lipid peroxidation, acute injury or chronic disease related injury,	Oxidative stress, host response to infection, induction of antioxidant defenses
Ethanol	Ppb	Gut bacterial metabolism of sugars	Gastrointestinal and liver function
Ethylene	Ppb	Lipid peroxidation involved in molecular signaling, acute injury or chronic disease-related injury	Oxidative stress, host response to infection, induction of antioxidant defenses
Hydrogen	Ppm	Gut bacterial metabolism of carbohydrates, lactase deficiency, disorders of di- and monosaccharide malabsorption, starch malabsorption	Gastroenteric diseases, disorders of digestion and absorption
Hydrogen cyanide	Ppb	Metabolic products of bacteria, synthesized by *P. aeruginosa*	Infections
Hydrogen sulfide	Ppb	Bacterial metabolism of thiol containing proteins mediator of brain	Gastrointestinal and liver function
Isoprene	Ppb	May be involved in regulation of HMGCoA reductase	Cholesterol biosynthesis; psychological stress
Leukotrienes	Ppb	Inflammatory processes	COPD
Isoprostanes	Ppb	Inflammatory processes	Cystic fibrosis, asthma, COPD
Methane	Ppm	Disaccharidase deficiency, gastrointestinal transit time, bacterial overgrowth, intestinal statis	Gastrointestinal diagnoses, gut bacterial metabolism of carbohydrates
Methanethiol	Ppb	Methionine metabolism	Hepatic function
Methylamine	Ppb	Protein metabolism	–
Methylnicotinate	Ppb	Metabolic products of bacteria	Infections
Methyl sulfide	Ppb	–	Hepatic function
Nitric oxide	Ppb	Pulmonary inflammation, production catalyzed by nitric oxide synthases involved in vasodilatation, or neurotransmission	Asthma, COPD, cystic fibrosis, pulmonary allograft dysfunction, lung cancer, host response to infection, induction of antioxidant defenses
Nitrite/nitrate	Ppb	Pulmonary inflammation	Cystic fibrosis, asthma
1-Pentane	Ppb	Lipid peroxidation, water % respiration	Oxidative stress, lipid peroxidation, acute injury or chronic disease related injury, host response to infection, induction of antioxidant defenses
2-Pentyl furan	Ppb	Metabolic products of bacteria	Infections
Prostanoids	Ppb	Pulmonary inflammation	COPD
Vinyl chloride	Ppb	–	VOCs exposure
Cis-1,2-dichloroethene	Ppb	–	VOCs exposure
Chloroform	Ppb	–	VOCs exposure
Bromodichloromethane	Ppb	–	VOCs exposure
Trichloroethene	Ppb	–	VOCs exposure
H_2O_2	Ppb	Oxidative stress	Asthma, COPD, bronchiectasis, ARDS
isotopes of carbon (^{13}C or ^{14}C)		*H. pilori* infection	Gastritis, duodenal ulcer, gastric ulcer, and gastric cancer
BMAC[a]	Ppb	Lipid peroxidation	Oxidative stress
CO_2/O_2 ratio	–	Excretion of drugs	Respiratory monitoring CO_2/O_2 ratio

[a] Breath methylated alkane contour.

Table 2
Unusual breath odors associated with disease or ingestion (modified from Hayden, G F., Olfactory diagnosis in medicine. Postgrad. Med. 1980, 67, 110-115).

General odor	Description of odor	Disease or offending substance
Sweet	Fruity; acetone-like; like decomposing apples	Ketoacidosis (e.g., diabetes or starvation), lacquer, chloroform, salicylates
	Fruity; alcoholic	Alcohol, phenol
	Fruity; pear-like; acrid, penetrating wintergreen	Chloral hydrate, paraldehyde methyl salicylate
	Aromatic; pungent	Ethchlorvynol
Fishy	Fishy	Uremia (trimethylamine)
	Fishy; rancid butter; boiled cabbage	Hypermethioninemia
Musty	Musty fish; raw liver; feculent; newmown clover (fetor hepaticus)	Hepatic failure (mercaptans, dimethyl sulfide)
Feculent	Feculent; foul	Intestinal obstruction, esophageal diverticulum
Urine-like	Ammoniacal	Uremia (ammonia)
Foul	Foul; putrid	Lung abscess, emphysema (especially anaerobic), intranasal foreign body
Halitosis	Severe 'bad breath'	Trench mouth (Vincent's angina), amphetamines
Other	Sweaty feet; cheesy	Isovaleric academia (odor-of-sweaty-feet syndrome)
	Bitter almond	Cyanide (e.g., apricot pits), jetberry bush
	Burned rope	Marijuana
	Camphor	Naphthalene (mothball pica)
	Coal gas (stove gas)	Carbon monoxide (odorless but associated with coal gas)
	Disinfectant	Phenol, creosote
	Garlic	Phosphorus, arsenic, tellurium, parathion, malathion

(Mazzatenta et al., 2013b). Thus, the measurement of VOCs enables to assess the level of central neural fatigue in both healthy and sick subjects. We suggest there is a biological plausibility of a future use of breath analysis to investigate the pathogenesis of genetic syndromes, e.g., Down's that is known to exhibit altered ethane production, or neurodegenerative diseases, e.g., Alzheimer's, by application of the breath real-time monitor in combination with cognitive performance. (Tables 1 and 2).

Conflicts of interest

The authors declare no conflicts of interest in relation to this article.

References

Aghdassi, E., Allard, J.P., 2000. Breath alkanes as a marker of oxidative stress in different clinical conditions. Free Radical Biology and Medicine 28, 880–886.

ATS/ERS, 2005. ATS/ERS recommendations for standardized procedures for the online and offline measurement of exhaled lower respiratory nitric oxide and nasal nitric oxide. American Journal of Respiratory and Critical Care Medicine 171, 912–930.

Amann, A., Miekisch, W., Pleil, J., Risby, T., Schubert, J., 2010. Methodological issues of sample collection and analysis of exhaled breath. In: Horvath, I., de Jongste, J.C. (Eds.), European Respiratory Monograph: Exhaled Biomarkers. pp. 96–114.

Ashok, B.T., Ali, R., 1999. The aging paradox: free radical theory of aging. Experimental Gerontology 34, 293–303.

Balseiro, S.C., Correia, H.R., 2006. Is olfactory detection of human cancer by dogs based on major histocompatibility complex-dependent odour components? A possible cure and a precocious diagnosis of cancer. Medical Hypotheses 66, 270–272.

Cao, W., Duan, Y., 2006. Breath analysis: potential for clinical diagnosis and exposure assessment. Clinical Chemistry 52, 800–811.

Carpagnano, G.E., 2011. Exhaled breath analysis and sleep. Journal of Clinical Sleep Medicine 7 (5), S34–S37.

Chen, S., Zieve, L., Mahadevan, V., 1970. Mercaptans and dimethyl sulfide in the breath of patients with cirrhosis of the liver. Effect of feeding methionine. Journal of Laboratory and Clinical Medicine 75, 628–635.

Christie, J.D., Edwards, L.B., Aurora, P., Dobbels, F., Kirk, R., Rahmel, A.O., Taylor, D.O., Kucheryavaya, A.Y., Hertz, M.I., 2008. Registry for the International Society for Heart and Lung Transplantation: twenty-fifth official adult lung and heart/lung transplantation report. Journal of Heart and Lung Transplantation 27, 943–956.

Clini, E., Bianchi, L., Pagani, M., Ambrosino, N., 1998. Endogenous nitric oxide in stable COPD patients: correlates with severity of disease. Thorax 53, 881–883.

Cope, K.A., Watson, M.T., Foster, W.M., Sehnert, S.S., Risby, T.H., 2004. Effects of ventilation on the collection of exhaled breath in humans. Journal of Applied Physics 96, 1371–1379.

Crofford, O.B., Mallard, R.E., Winton, R.E., Rogers, N.L., Jackson, J.C., Keller, U., 1977. Acetone in breath and blood. Transactions of the American Clinical and Climatological Association 88, 128–139.

Dahl, M., Nordestgaard, B.J., 2009. Markers of early disease and prognosis in COPD. International Journal of COPD 4, 157–167.

Dannecker, J.R., Shaskan, E.G., Phillips, M., 1981. A new highly sensitive assay for breath acetaldehyde: detection of endogenous levels in humans. Analytical Biochemistry 114, 1–7.

Di Natale, C., Macagnano, A., Martinelli, E., Paolesse, R., D'Arcangelo, G., Roscioni, C., Finazzi-Agrò, A., D'Amico, A., 2003. Lung cancer identification by the analysis of breath by means of an array of non-selective gas sensors. Biosensors and Bioelectronics 18, 1209–1218.

Dumelin, E.E., Tappel, A.L., 1977. Hydrocarbon gases produced during in vitro peroxidation of polyunsaturated fatty acids and decomposition of preformed hydroperoxides. Lipids 12, 894–900.

Dweik, R.A., Boggs, P.B., Erzurum, S.C., Irvin, C.G., Leigh, M.W., Lundberg, J.O., Olin, A.C., Plummer, A.L., Taylor, D.R., 2011. An official ATS clinical practice guideline: interpretation of exhaled nitric oxide levels (FeNO) for clinical applications. American Journal of Respiratory and Critical Care Medicine 184, 60615.

Fisher, A.J., Gabbay, E., Small, T., Doig, S., Dark, J.H., Corris, P.A., 1998. Cross sectional study of exhaled nitric oxide levels following lung transplantation. Thorax 53, 454–458.

Gendron, K.B., Hockstein, N.G., Thaler, E.R., Vachani, A., Hanson, C.W., 2007. In vitro discrimination of tumor cell lines with an electronic nose. Otolaryngology – Head and Neck Surgery 137, 269–273.

Hadjiliadis, D., Davis, R.D., Steele, M.P., Messier, R.H., Lau, C.L., Eubanks, S.S., Palmer, S.M., 2003. Gastro-esophageal reflux disease in lung transplant recipients. Clinical Transplantation 17, 363–368.

Henderson, M.J., Karger, B.A., Wren Shall, G.A., 1952. Acetone in the breath: a study of acetone exhalation in diabetic and nondiabetic human subjects. Diabetes 1, 188–193.

Horvath, I., Hunt, J., Barnes, P.J., on behalf of the ERS/ATS Task Force, 2005. Exhaled breath condensate: methodological recommendations and unresolved questions. European Respiratory Journal 26, 523–548.

Horvath, I., Lazar, Z., Gyulai, N., Kollai, M., Losonczy, G., 2009. Exhaled biomarkers in lung cancer. European Respiratory Journal 34, 261–275.

Hunt, J., 2002. Exhaled breath condensate: an evolving tool for noninvasive evaluation of lung disease. Journal of Allergy and Clinical Immunology 110, 28–34.

Jansson, B.O., Larsson, B.T., 1969. Analysis of organic compounds in human breath by gas chromatography–mass spectrometry. Journal of Laboratory and Clinical Medicine 74, 961–966.

Lases, E.C., Duurkens, V.A.M., Gerritsen, W.B., Haas, F.J., 2000. Oxidative stress after lung resection therapy: a pilot study. Chest 117, 999–1003.

Machado, R.F., Laskowski, D., Deffenderfer, O., Burch, T., Zheng, S., Mazzone, P.J., Mekhail, T., Jennings, C., Stoller, J.K., Pyle, J., Duncan, J., Dweik, R.A., Erzurum, S.C., 2005. Detection of lung cancer by sensor array analyses of exhaled breath. American Journal of Respiratory and Critical Care Medicine 171, 1286–1291.

Mathers, C.D., Loncar, D., 2006. Projections of global mortality and burden of disease from 2002 to 2030. PLoS Medicine 3, e442.

Mazzatenta, A., Pokorski, M., Cozzutto, S., Barbieri, P., Veratti, V., Di Giulio, C., 2013a. Non-invasive assessment of exhaled breath pattern in patients with multiple chemical sensibility disorder. Advances in Experimental Medicine and Biology 756, 179–188.

Mazzatenta, A., Pokorski, M., Di Giulio, C., 2013b. Real-time breath analysis in type 2 diabetes mellitus patients during cognitive task. Advances in Experimental Medicine and Biology 788, Chapter 35, in press.

Mazzone, P.J., Hammel, J., Dweik, R., Na, J., Czich, C., Laskowski, D., Mekhail, T., 2007. Lung cancer diagnosis by the analysis of exhaled breath with a colorimetric sensor array. Thorax 62, 565–568.

Metidiewa, D., Koska, C., 2000. Reactive oxygen species and reactive nitrogen species: relevance to cyto(neuro)toxic events and neurological disorders: an overview. Neurotoxicity Research 1, 197–233.

Miekisch, W., Schubert, J.K., Noeldge-Schomburg, G.F., 2004. Diagnostic potential of breath analysis: focus on volatile organic compounds. Clinica Chimica Acta 347, 25–39.

Montag, S., Frank, M., Ulmer, H., Wernet, D., Göpel, W., Rammensee, H.G., 2001. "Electronic nose" detects major histocompatibility complex-dependent prerenal and postrenal odor components. Proceedings of the National Academy of Sciences of the United States of America 98, 9249–9254.

Montuschi, P., 2005. Exhaled breath condensate analysis in patients with COPD. Clinica Chimica Acta 356, 22–34.

Montuschi, P., Kharitonov, S.A., Ciabattoni, G., Corradi, M., van Rensen, L., Geddes, D.M., Hodson, M.E., Barnes, P.J., 2000. Exhaled 8-isoprostane as a new non-invasive biomarker of oxidative stress in cystic fibrosis. Thorax 55, 205–209.

Olin, A.C., Rosengren, A., Thelle, D.S., Lissner, L., Bake, B., Toren, K., 2006. Height, age, and atopy are associated with fraction of exhaled nitric oxide in a large adult general population sample. Chest 130, 1319–1325.

Orens, J.B., Garrity Jr., E.R., 2009. General overview of lung transplantation and review of organ allocation. Proceedings of the American Thoracic Society 6, 13–19.
Pauling, L., Robinson, A.B., Teranishi, R., Cary, P., 1971. Quantitative analysis of urine vapor and breath by gas-liquid partition chromatography. Proceedings of the National Academy of Sciences of the United States of America 68, 2374–2376.
Perman, J.A., 1991. Clinical application of breath hydrogen measurements. Canadian Journal of Physiology and Pharmacology 69, 111–115.
Phillips, M., 1992. Breath tests in medicine. Scientific American 267, 74–79.
Phillips, M., 1997. Method for the collection and assay of volatile organic compounds in breath. Analytical Biochemistry 247, 272–278.
Phillips, M., Cataneo, R.N., Cheema, T., Greenberg, J., 2004. Increased breath biomarkers of oxidative stress in diabetes mellitus. Clinica Chimica Acta 344, 189–194.
Phillips, M., Cataneo, R.N., Cummin, A.R.C., Gagliardi, A.J., Gleeson, K., Greenberg, J., Maxfield, R.A., Rom, W.N., 2003. Detection of lung cancer with volatile markers in the breath. Chest 123, 2115–2123.
Phillips, M., Herrera, J., Krishnan, S., Zain, M., Greenberg, J., Cataneo, R.N., 1999. Variation in volatile organic compounds in the breath of normal humans. Journal of Chromatography B: Biomedical Sciences and Applications 729, 75–88.
Radhika, V., Proikas-Cezanne, T., Jayaraman, M., Onesime, D., Ha, J.H., Dhanasekaran, D.N., 2007. Chemical sensing of DNT by engineered olfactory yeast strain. Nature Chemical Biology 3, 325–330.
Riely, C.A., Cohen, G., Lieberman, M., 1974. Ethane evolution: a new index of lipid peroxidation. Science 183, 208–210.
Risby, T.H., 2002. Volatile organic compounds as markers in normal and diseased states. In: Marczin, N., Yacoub, M. (Eds.), Disease Markers in Exhaled Breath: Basic Mechanisms and Clinical Applications. NATO ASI Series. IOS Press, Amsterdam, pp. 113–122.
Risby, T.H., Sehnert, S.S., 1999. Clinical application of breath biomarkers of oxidative stress status. Free Radical Biology and Medicine 27, 1182–1192.
Risby, T.H., Solga, S.F., 2006. Current status of clinical breath analysis. Applied Physics B 85, 421–426.
Romagnuolo, J., Schiller, D., Bailey, R.J., 2002. Using breath tests wisely in a gastroenterology practice: an evidence-based review of indications and pitfalls in interpretation. American Journal of Gastroenterology 97, 1113–1126.
Rosias, P.P.R., Dompeling, E., Dentener, M.A., Pennings, H.J., Hendriks, H.J., Van Iersel, M.P., Van Iersel, M.P., Jöbsis, Q., 2004. Childhood asthma: exhaled markers of airway inflammation, asthma control score, and lung function tests. Pediatric Pulmonology 38, 107–114.
Skeldon, K.D., McMillan, L.C., Wyse, C.A., Monk, S.D., Gibson, G., Patterson, C., France, T., Longbottom, C., Padgett, M.J., 2006. Application of laser spectroscopy for measurement of exhaled ethane in patients with lung cancer. Respiratory Medicine 100, 300–306.
Smith, D., Spanel, P., Davies, S., 1999. Trace gases in breath of healthy volunteers when fasting and after a protein-calorie meal: a preliminary study. Journal of Applied Physiology 87, 1584–1588.
Solga, S.F., Risby, T.H., 2010. What is normal breath? Challenge and opportunity. IEEE Sensors Journal 10, 7–9.
Spanel, P., Smith, D., 2008. Quantification of trace levels of the potential cancer biomarkers formaldehyde, acetaldehyde and propanol in breath by SIFT-MS. Journal of Breath Research 2, 046003.
Sysoev, V.V., Button, B.K., Wepsiec, K., Dmitriev, S., Kolmakov, A., 2006. Toward the nanoscopic "electronic nose": hydrogen versus carbon monoxide discrimination with an array of individual metal oxide nano- and mesowire sensors. Nano Letters 6, 1584–1588.
Szefler, S.J., Martin, R.J., King, T.S., Boushey, H.A., Cherniack, R.M., Chinchilli, V.M., Craig, T.J., Dolovich, M., Drazen, J.M., Fagan, J.K., Fahy, J.V., Fish, J.E., Ford, J.G., Israel, E., Kiley, J., Kraft, M., Lazarus, S.C., Lemanske Jr., R.F., Mauger, E., Peters, S.P., Sorkness, C.A., 2002. Asthma Clinical Research Network of the National Heart Lung, and Blood Institute. Significant variability in response to inhaled corticosteroids for persistent asthma. Journal of Allergy and Clinical Immunology 109, 410–418.
Szefler, S.J., Wenzel, S., Brown, R., Erzurum, S.C., Fahy, J.V., Hamilton, R.G., Hunt, J.F., Kita, H., Liu, A.H., Panettieri, R.A., Schleimer, R.P., Minnicozzi, M., 2012. Asthma outcomes: biomarkers. Journal of Allergy and Clinical Immunology 129, S9–S23.
Thorpe, M.J., Balslev-Clausen, D., Kirchner, M.S., Ye, J., 2008. Cavity enhanced optical frequency comb spectroscopy: application to human breath analysis. Optics Express 18, 2387–2397.
Vidic, J.M., Grosclaude, J., Persuy, M.A., Aioun, J., Salesse, R., Pajot-Augy, E., 2006. Quantitative assessment of olfactory receptors activity in inmobilized nanosomes: a novel concept for bioelectronic nose. Lab on a Chip 6, 1026–1032.
Williams, H., Pembroke, A., 1989. Sniffer dogs in the melanoma clinic? Lancet 1, 734.