Hypothesis/Commentary

Strengths, Weaknesses, and Opportunities of Diagnostic Breathomics in Pleural Mesothelioma—A Hypothesis

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Abstract

Past and present asbestos use will reflect in increasing numbers of mesothelioma cases in the next decades, diagnosed at a late stage and with a dismal prognosis. This stresses the need for early detection tools, which could improve patients’ survival. Recently, breath analysis as a noninvasive and fast diagnostic tool has found its way into biomedical research. High-throughput breathomics uses spectrometric, chromatographic, and sensor techniques to diagnose asbestos-related pulmonary diseases based upon volatile organic compounds (VOC) in breath. This article reviews the state-of-the-art available breath analyzing techniques and provides the insight in the current use of VOCs as early diagnostic or prognostic biomarkers of mesothelioma to stimulate further research in this field. Cancer Epidemiol Biomarkers Prev; 23(6); 898–908. ©2014 AACR.

Introduction

Asbestos fibers cause malignant pleural mesothelioma (MPM), an aggressive tumor from the serosal surface lining the pleural cavity (1). Because of the large asbestos consumption in the past, MPM incidence rates will further increase the next decade (2, 3), although with large intercountry differences (4, 5), and peak between 2015 and 2020 (1, 6). This MPM epidemic will cost the United States and the European Union $200 billion and $80 billion, respectively, in compensation, urging the need for an early detection (7).

The “Holy Grail” in MPM diagnosis would be a noninvasive, accurate test in asbestos-exposed persons at risk for developing MPM which makes early-stage detection possible, reduces the economic burden of wild screening, and improves MPM management. Present research efforts have, however, not yet revealed a validated diagnostic blood biomarker (5, 8).

Breathomics is an innovative, noninvasive tool that uses high-throughput technologies to identify organic molecules in breath as biomarkers that reflect the patient’s metabolic status. The aim of this manuscript is to critically review the current state of the art concerning different techniques used for gaseous breath analysis, focused on their diagnostic yield in various asbestos-related diseases and MPM specifically to stimulate future MPM biomarker research using breathomics and improve MPM management.

Search Strategy and Literature Selection

MEDLINE (PubMed) and Web of Science were searched for studies concerning exhaled breath research in asbestos-related pulmonary diseases until December 2013. Following keywords were selected as MeSH terms and used as search terms in PubMed and Web of Science: "asbestos," "asbestosis," "mesothelioma," "lung neoplasms," and "breath tests." Other additional search terms included "breath analysis," "pleural plaques," "pleural thickening," "pleural effusion," "volatile organic compounds" (VOC), and "lung cancer." After a first inspection of the literature "electronic nose" (eNose), "ion mobility spectrometry" (IMS), "gas chromatography-mass spectroscopy" (GC-MS), "exhaled breath condensate" (EBC) and "dog" were added as additional terms for specific search combinations. Only literature published in English on human material was considered. References of the 131 selected publications were searched manually for additional relevant literature concerning breath analyzing techniques in asbestos-related diseases. This delivered another 21 publications.

The Spectrum of Asbestos-Related Diseases

Asbestos fibers are hydrated silicate minerals containing a high tensile strength and resistance to chemical and thermal degradation, making these “magic minerals” interesting for insulation in (ship) building (9). Hence, many people were professionally exposed to the fibers. Inhaling asbestos fibers damages the lung parenchyma and the pleura, leading not only to benign conditions like pleural effusions, pleural plaques, diffuse pleural thickening (DPT), and asbestosis, but also to malignant diseases like lung cancer (although in association with tobacco) and MPM (Fig. 1). The World Health Organization estimates that annually 125,000 new persons are confronted with an occupational asbestos exposure (10) and worldwide, there are approximately 25,000 patients diagnosed with mesothelioma per year (11). The lifetime MPM...
risk after occupational asbestos exposure is 10% (7) with a mean latency period between exposure and diagnosis of 40 years (range 15–67 years; ref. 6). A nonspecific symptomatology and a lack of imaging accuracy delay its diagnosis so that patients present in an advanced stage which jeopardizes potential curative management (1). Despite that current guidelines dissuade screening for MPM (7), asbestos-exposed individuals are wildly and widely subjected to nonspecific investigations [lung function testing, chest X-rays, and computed tomography (CT) scans] at an unknown cost (6).

Asbestos exposure also increases the likelihood for lung cancer development through a synergistic effect with smoking and via the mechanism of fibrosis (12, 13). Any biomarker of asbestos-induced lung cancer is hence likely to be confounded by smoking. Happily, a low-dose spiral CT scan has recently been advocated an effective technique for the early diagnosis of lung cancer (14, 15) but not of mesothelioma (16, 17), urging the need to search for other early detection tools for the latter disease.

Breath Analysis: Techniques and Applications

Exhaled breath is easily and noninvasively retrievable, resembles the arterial concentrations of biologic substances, and can be monitored in real time. It consists of a liquid phase containing water and proteins and a
gaseous phase containing oxygen, nitrogen, carbon dioxide, water, inert gases, VOCs, and non-VOCs (18–20). Since their first discovery, more than 3,000 VOCs have been detected in breath (21), arising from exogenous sources (via inhalation or skin adsorption) or from endogenous biochemical processes (via oxidative stress and fat metabolism) and are catabolized through cytochrome P450 enzymes. Independent of their origin, VOCs are transported through the blood to the lungs where they enter the breath by the alveolar gas exchange mechanisms. Hence, changes in the VOC metabolism result in different VOC profiles in breath (22).

Oxidative stress plays a key role in endogenous VOC production: acetone is linked to lipolysis via decarboxylation of excess acetyl-CoA after oxidation of fatty acids, isoprene is liberated during the mevalonic pathway of cholesterol synthesis, and hydrocarbons are formed through lipid peroxidation of polyunsaturated fatty acids in the cellular membranes (23). Because asbestos-related diseases are driven by oxidative stress and inflammation, markers of oxidative damage, such as VOCs, are hence likely to be altered. Asbestos fibers have indeed a high iron content which directly induces oxidative stress generating reactive oxygen species (ROS) and nitrogen species (RNS; refs. 24, 25). Because of the asbestos fiber’s high length/width ratio, alveolar macrophages fail to engulf them, leading to a “frustrated phagocytosis” (ref. 26; Fig. 1). In this way, asbestos indirectly induces the release of oxidants, cytokines, and growth factors by the macrophages (and to a lesser extent by the mesothelial cells). This sustains the relentless generation of ROS and RNS which lead to (i) lipid peroxidation generating saturated hydrocarbons and aldehydes, (ii) protein oxidation, and (iii) mutagenic DNA lesions through the generation of 8-oxo-7,8-dihydro-2′-deoxyguanosine and 8-nitroguanine (27, 28). In addition, asbestos-induced mesothelial cell death is also linked to the inflammatory reaction associated with asbestos carcinogenesis (29) and hence could play a role in VOC production. When asbestos damages the mesothelial cells, High Mobility Group Box-1 (HMGB1) is released, inducing the accumulation of macrophages, inflammation and the release of TNF-α (30, 31), which binds its induced receptor on the mesothelial cells. This activates the NF-κB pathway, increasing the survival of damaged mesothelial cells and aiding MPM pathogenesis (29).

Several very sensitive breath analyzing techniques are now available for the liquid or gaseous phase of the breath and measure one of the different (non) volatile breath compounds or recognize VOC patterns.

**Analysis of the liquid phase**

Exhaled breath forms a condensate with water representing more than 99% of the EBC sample and contains different molecules ranging from simple ions to DNA (32, 33). Although not the scope of this review, diagnostic markers of asbestosis, pleural plaques, and DPT in EBC have been investigated (Table 1). The nonvolatile oxidative markers hydrogen peroxide (H_{2}O_{2}), 8-isoprostane, 4-hydroxy-trans-2-nonenal (HNE), 8-Hydroxy-2′-Deoxyguanosin (8-OHdG), and the leukotrienes (LT) B4, D4 and E4 were found increased in the breath of asbestos-exposed individuals or patients with asbestos-related diseases compared with healthy controls (34–41). However, no EBC measurements have been performed for MPM specifically. LTB4 is a potent chemotactic factor for neutrophils and asbestos exposure has been shown to provoke LTB4 secretion in alveolar macrophages in vivo (42, 43). These findings suggest that the LTB4 level in EBC reflects the inflammatory response to asbestos and is attractive for further research in MPM. The EBC level of 8-isoprostane is related to the degree of oxidative stress, tissue damage, and fibrosis, and hence, could also be used as a marker for lung cancer or could be confounded by smoking.

**Analysis of the gaseous phase**

Endogenous volatile metabolic products derived from the tumor or its environment and present in the gaseous phase of breath are of interest because they reflect tumorspecific biomarkers that are linked to their pathogenesis. Nitric oxide (NO) serves as a signaling molecule maintaining physiologic control of airway function (44). Asbestos induces chronic lung inflammation in which an inducible form of NO-synthase is upregulated and the pulmonary gas diffusion capacity is decreased. Hence, the fractional exhaled NO (FeNO) in the gaseous phase of the breath of asbestos-exposed individuals increases. This was seen in patients with asbestosis compared with those with pleural plaques, DPT, and healthy controls (45; Table 1). When flow rates over 250 mL/minutes are achieved, alveolar NO tends to be higher in patients with asbestosis (34, 36) and in asbestos-exposed persons with borderline high-resolution CT parenchymal changes (41). This reflects the ongoing lower respiratory tract inflammation and it seems that alveolar NO is related to the degree of pulmonary fibrosis rather than to the asbestos exposure per se. Hence, the increased production of NO is not tumor specific, is attributed to tumor-associated nonspecific immunologic and (asbestos-related) inflammatory mechanisms, and correlates with the intensity of NO-synthase 2 expression in alveolar macrophages (46).

Although NO levels are dependent of age, sex, and lung function, FeNO determination in early MPM diagnosis can have a role that has not been investigated until today. The most popular technique in determining FeNO is chemiluminescence, although handheld devices with electrochemical sensors are available, such as NIOX MINO (AeroCrine AB).

Advances in detection systems resulted in (i) combinations of mass spectrometers and fast flow tubes such as proton-transfer-reaction mass spectrometry and selected ion flow tube MS, (ii) spectroscopic methods for identification and quantification of small molecules such as IMS, and (iii) nonspecific eNoses that “smell” gas combinations. All these methods provide the capability of easily gathering repeated samples and obtaining results immediately in a relatively short period of time.
Gas chromatography-mass spectrometry. GC-MS detects and quantifies gases from 100 parts per million to 1 part per billion by separating compounds based upon their elution times from the GC-column and characteristic fragmentation pattern obtained by MS (Fig. 2A). Today, only one group reported GC-MS analysis of the breath of, respectively, 13 patients with MPM, 13 healthy asbestos-exposed individuals, and 13 healthy controls (47). Table 2 summarizes the 18 VOCs identified as being able to discriminate MPM breath from controls with most of them being alkanes. The authors found higher cyclohexane concentrations in the breath of patients with MPM compared with the other control groups in which a model they have built distinguished these groups with 97% accuracy.

<table>
<thead>
<tr>
<th>Marker</th>
<th>Disease (n)</th>
<th>Control subjects (n)</th>
<th>Method</th>
<th>Result</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Oxidative stress</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>H₂O₂</td>
<td>Asbestosis (18)</td>
<td>Healthy (26)</td>
<td>EcoScreen + EIA</td>
<td>Asbestosis &gt; HC</td>
<td>(34)</td>
</tr>
<tr>
<td>Pleural plaques (26)</td>
<td>Healthy (26)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DPT (16)</td>
<td>Healthy (26)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8-isoprostane</td>
<td>Asbestos-exposed (92)</td>
<td>Healthy (46)</td>
<td>SPE + LC-ESI-MS/MS</td>
<td>Asbestos-exposed &gt; HC</td>
<td>(38)</td>
</tr>
<tr>
<td>Asbestos-exposed (44)</td>
<td>NA</td>
<td></td>
<td>SPE + LC-ESI-MS/MS</td>
<td>Asbestos-exposed &gt; HC</td>
<td>(40)</td>
</tr>
<tr>
<td>Asbestos-exposed (45)</td>
<td>Healthy (29)</td>
<td>SPE + LC-ESI-MS/MS</td>
<td>Asbestos-exposed &gt; HC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Asbestosis (15)</td>
<td>Healthy (15)</td>
<td>EcoScreen + EIA</td>
<td>Asbestosis &gt; HC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pleural plaques (26)</td>
<td>Healthy (26)</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>DPT (16)</td>
<td>Healthy (26)</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Asbestos-exposed (66)</td>
<td>Healthy, nonexposed (41)</td>
<td>Chemoluminescence NO analyzer</td>
<td>Asbestos-exposed &gt; HC</td>
<td></td>
<td>(41)</td>
</tr>
<tr>
<td>HNE</td>
<td>Asbestos-exposed (45)</td>
<td>Healthy (29)</td>
<td>SPE + LC-ESI-MS/MS</td>
<td>Asbestos-exposed &gt; HC</td>
<td>(39)</td>
</tr>
<tr>
<td>8-OHdG</td>
<td>Asbestosis (10)</td>
<td>Healthy (10)</td>
<td>Lyophilization + LC-ESI-MS/MS</td>
<td>Asbestosis &gt; HC</td>
<td>(35)</td>
</tr>
<tr>
<td>FeNO</td>
<td>Asbestosis (12)</td>
<td>Healthy (35)</td>
<td>Chemoluminescence NO analyzer</td>
<td>Asbestosis &gt; HC</td>
<td>(45)</td>
</tr>
<tr>
<td>Pleural plaques (32)</td>
<td>Healthy (35)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DPT (12)</td>
<td>Healthy (35)</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Asbestosis (15)</td>
<td>Healthy (15)</td>
<td>Chemoluminescence NO analyzer</td>
<td>No differences</td>
<td></td>
<td>(36)</td>
</tr>
<tr>
<td>Asbestos-exposed (66)</td>
<td>Healthy, nonexposed (41)</td>
<td>Chemoluminescence NO analyzer</td>
<td>Asbestos-exposed &gt; HC</td>
<td></td>
<td>(41)</td>
</tr>
<tr>
<td><strong>Lipid peroxidation</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LTB₄</td>
<td>Asbestosis (45)</td>
<td>Healthy (27)</td>
<td>SPE + LC-ESI-MS/MS</td>
<td>Asbestosis &gt; HC</td>
<td>(37)</td>
</tr>
<tr>
<td>Silicosis (37)</td>
<td></td>
<td></td>
<td></td>
<td>Asbestosis &gt; Silicosis</td>
<td></td>
</tr>
<tr>
<td>Asbestosis (15)</td>
<td>Healthy (15)</td>
<td>EcoScreen condensor + EIA</td>
<td>Asbestosis &gt; HC</td>
<td></td>
<td>(36)</td>
</tr>
<tr>
<td>Asbestos-exposed (66)</td>
<td>Healthy, nonexposed (41)</td>
<td>EcoScreen condensor + EIA</td>
<td>Asbestos-exposed &gt; HC</td>
<td></td>
<td>(41)</td>
</tr>
<tr>
<td>LTC₄, D₄, E₄</td>
<td>Asbestosis (45)</td>
<td>Healthy (27)</td>
<td>SPE + LC-ESI-MS/MS</td>
<td>Asbestosis &gt; HC</td>
<td>(37)</td>
</tr>
<tr>
<td>Silicosis (37)</td>
<td></td>
<td></td>
<td></td>
<td>Silicosis &gt; HC</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: 8-isoprostane, 8-iso-prostaglandine F₂α; EIA, enzyme immunoassay; HC, healthy control; NA, not applicable.
Figure 2. A, general principle of GC-MS. The breath is collected into bags (Tedlar, Mylar, Polytetrafluoroethylene) and VOCs are subsequently concentrated by adsorbing them onto a cold surface (via EBC devices, cryofocussing or lyophilization) or onto some adsorbents (Tenax columns) from which they are released into the sample inlet of the GC-MS via solid phase microextraction or thermal desorption. A carrier flow gas takes the sample over a heated GC column to separate the VOCs based upon their chemical characteristics. When entering the MS, samples are ionized, accelerated, and led to a detector through time-of-flight or quadrupole analysis. B, principles of IMS. Breath samples are thermally desorbed or enter the IMS through direct sampling via an MCC to separate the different chemicals based upon their chemical characteristics and from the water content of the breath. In general, nitrogen or synthetic air is used as a carrier gas of which the molecules are directly ionized by the β-particles of a 63Ni radioactive source. Positive carrier gas ions (reactant ions) and free electrons will become available and undergo different chemical reactions with the breath analyte ions to form product ions. After the ionization phase, a short opening of an ion shutter (e.g., Bradbury–Nielsen gate) every 100 to 300 ms for 300 ms allows the ionized breath compounds to enter a drift tube (10–15 cm) which separates the VOCs based upon their ion mobilities (charge, mass, size, and shape). In the drift tube, VOCs move to a detector (e.g., Faraday plate) under the influence of a low electric field and a counter gas which generates a small electric current. The time after opening of the grid and the arrival at the detector (the drift time) is measured to characterize the ions. C, example of a smellprint. Subjects need to breath normally for 5 minutes through a three-way non-rebreathing valve with a VOC and a silica filter. After a maximal deep inspiration, subjects exhale a single vital capacity volume into a 10-L Tedlar bag connected to the expiratory port and silica reservoir, sampling all of the breath. Next, the Tedlar bag is connected to the eNose for analysis or the sample is first brought onto Tenax columns within 10 minutes and thermally desorbed and subsequently sampled in the eNose. The bulk of the VOCs change the resistance of the 32 polymer carbon sensors of the Cyranose 320. The change in relative resistance (ΔR/R) for 32 carbon polymer sensors is known as a smellprint.
### Table 2. Volatile breath biomarkers measured with GC-MS in patients with MPM and lung cancer

<table>
<thead>
<tr>
<th>VOC</th>
<th>Controls (n)</th>
<th>MPM (n)</th>
<th>Lung cancer (n)</th>
<th>Method</th>
<th>Sample</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetophenone</td>
<td>13</td>
<td>13</td>
<td>TD-GC-MS</td>
<td>Breath</td>
<td>(47)</td>
<td></td>
</tr>
<tr>
<td>Benzene, trimethyl-</td>
<td>13</td>
<td>13</td>
<td>TD-GC-MS</td>
<td>Breath</td>
<td>(47)</td>
<td></td>
</tr>
<tr>
<td>Decane</td>
<td>13</td>
<td>13</td>
<td>TD-GC-MS</td>
<td>Breath</td>
<td>(47)</td>
<td></td>
</tr>
<tr>
<td>Heptane</td>
<td>13</td>
<td>13</td>
<td>TD-GC-MS</td>
<td>Breath</td>
<td>(47)</td>
<td></td>
</tr>
<tr>
<td>Heptane, 2,4-dimethyl-</td>
<td>48 (NH)</td>
<td>60</td>
<td>TD-GC-MS</td>
<td>Breath</td>
<td>(47)</td>
<td></td>
</tr>
<tr>
<td>1-Hexanol, 2-ethyl-</td>
<td>13</td>
<td>13</td>
<td>TD-GC-MS</td>
<td>Breath</td>
<td>(47)</td>
<td></td>
</tr>
<tr>
<td>Limonene</td>
<td>13</td>
<td>13</td>
<td>TD-GC-MS</td>
<td>Breath</td>
<td>(47)</td>
<td></td>
</tr>
<tr>
<td>Nonane, dimethyl-</td>
<td>13</td>
<td>13</td>
<td>TD-GC-MS</td>
<td>Breath</td>
<td>(47)</td>
<td></td>
</tr>
<tr>
<td>Octane, 3-methyl-</td>
<td>48 (NH)</td>
<td>60</td>
<td>TD-GC-MS</td>
<td>Breath</td>
<td>(47)</td>
<td></td>
</tr>
<tr>
<td>1,2-Pentadiene</td>
<td>13</td>
<td>13</td>
<td>TD-GC-MS</td>
<td>Breath</td>
<td>(47)</td>
<td></td>
</tr>
<tr>
<td>α-Pinene</td>
<td>13</td>
<td>13</td>
<td>TD-GC-MS</td>
<td>Breath</td>
<td>(47)</td>
<td></td>
</tr>
<tr>
<td>Styrene</td>
<td>48 (NH)</td>
<td>60</td>
<td>TD-GC-MS</td>
<td>Breath</td>
<td>(47)</td>
<td></td>
</tr>
<tr>
<td>Toluene</td>
<td>13</td>
<td>13</td>
<td>TD-GC-MS</td>
<td>Breath</td>
<td>(47)</td>
<td></td>
</tr>
</tbody>
</table>

(Continued on the following page)
However, cyclohexane and several other VOCs were also found in the breath or urine of patients with lung cancer and in in vitro studies with sensitivities and specificities ranging, respectively, from 71% to 86% and 66% to 100% (refs. 48–58; Table 2). This indicates that these VOCs originate from oxidative stress in the inflamed stroma and hence, lack specificity for MPM because they are also seen in other cancers like lung cancer. Because asbestos can also induce lung cancer (59), nonspecific inflammation-related VOCs in patients with lung cancer can be of great interest to investigate in MPM and vice-versa. Two reports identified 2-methylpentane as a potential marker for lung cancer (57, 58) which was also found in in vitro tests (60). Increased concentrations of C₄-C₂₄ hydrocarbons (61, 62) and C₃-C₉ aldehydes (63) reflecting oxidative stress and lipid peroxidation were also found in patients with lung cancer and represent also promising markers as inflammation-related VOCs in MPM.

**Ion mobility spectrometry.** IMS allows separation of VOCs in the breath by their ion mobilities (size, mass, shape, and charge; refs. 64, 65). Gaseous metabolites are firstly ionized in an ionization chamber by a low energetic ⁶⁷Ni β-radioactive source, although other sources exist such as UV light, lasers, electrical discharges, or electro-spray, followed by a separation in a drift tube under influence of a counter gas (Fig. 2B). Conventional IMS is based on absolute ion mobilities measured at low electric fields, whereas other IMS-based methods, as differential IMS or field asymmetric waveform IMS, use the mobility difference at high and low electrical field, eliciting a periodic asymmetric waveform (66). Combining IMS with multicapillary GC-columns (MCC) permits an effective preseparation of VOCs based upon their chemical characteristics before entering the ionization chamber and drift tube (Fig. 2B). A direct online measurement using 10 mL of human breath and a total analysis time of 10 to 15 minutes make MCC/IMS utterly suitable for clinical applications.

Data from MCC/IMS in patients with MPM or asbestos-related disease have presently not been reported. Nevertheless, its potential is demonstrated in three studies reporting breath analysis in patients with lung cancer. Westhoff and colleagues identified 23 peak regions discriminating patients with lung cancer from healthy controls with 0% error rate and a positive (PPV) and negative (NPV) predictive value of 100%, independently of smoking status, tumor-node-metastasis stage, and histology (65). However, their patients with lung cancer were older and at an advanced stage and the healthy controls were not age-matched. Another study compared bronchoscopically obtained tumor-side air with nontumor-side air wherein n-dodecane discriminated patients with lung adenocarcinoma from controls with 100% sensitivity, 75% specificity, 80% PPV, and 100% NPV (67). 2-butanol or 2-methylfuran and nonanal discriminated squamous cell carcinoma from healthy controls with sensitivities of 78% to 79%, specificities of 67% to 78%, PPVs of 70% to 80%, and NPVs of 75% to 88%, which was confirmed more recently (68). Although each patient with a tumor was its own control to minimize confounding and healthy subjects were not included, 2-butanol and nonanal had a higher value in the tumor-bearing lung but no correlation of the VOCs with the location or the stage of the tumor was found.

**Electronic noses.** eNoses are based upon the change in surface conductivity of nonselective sensors when exposed to a bulk of different volatile breath compounds. These detectors recognize breath patterns without identifying and quantifying these compounds. VOCs induce a physical or chemical sensor change that sends a signal to a computer which then makes a classification. By analyzing the breath of several patient groups and building a model based upon several data mining techniques, eNoses can be trained to identify a mixture of VOCs as a “smellprint” (Fig. 2C). By generating a database of breath signatures, identification of subjects with similar breath chemical characteristics becomes possible. Different sensors can be used as eNose sensors (69). The most frequently used eNose, the Cytano 320 (Intelligent Optical Systems, Inc.), is a commercial and portable system whose detection capacity consists of an array of 32 individual polymer sensors blended with carbon black composite.

Dragonei and colleagues (70) were the first to describe the use of an eNose in diagnosing MPM. They distinguished 13 patients with MPM from 13 asbestos-exposed individuals and 13 healthy controls with, respectively,
92% sensitivity and 86% and 69% specificity. This study was repeated, now distinguishing 20 patients with MPM from 18 asbestos-exposed and 42 healthy individuals with 90% sensitivity and 88% specificity (71).

An in vitro study used an eNose to compare the headspace between cell lines for mesothelioma, non-small cell lung cancer, metastatic squamous cell carcinoma, and healthy controls (72). eNoses separated the different malignant cell lines and discriminated malignant from nonmalignant cell lines. These two studies underline the potential of eNose analysis, next to GC-MS analysis, for detecting and diagnosing MPM.

Canines. Because eNose pattern recognition is based upon natural olfactory perception, dogs have been used to discriminate different breath patterns. Because of their sensitive smelling ability and learning capacity, several dog stocks were trained to recognize and discriminate different breath samples. Although no studies using canine scent for asbestosis or mesothelioma are available, patients with lung cancer have been distinguished from controls with a 71% to 83% sensitivity and 82% to 93% specificity (73, 74). However, bias in these studies result from confounding smoking, drugs, or the use of different dog stocks.

Table 3. Overview of breath analyzing techniques for volatile compounds

<table>
<thead>
<tr>
<th>GC-MS</th>
<th>eNose</th>
<th>IMS</th>
<th>Canine scent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitive (ppb-ppt)</td>
<td>No Specific VOC identification</td>
<td>Sensitive (ppt)</td>
<td>Time consuming (dog training)</td>
</tr>
<tr>
<td>Identification, detection, quantification of VOCs</td>
<td>Black box</td>
<td>VOC identification possible with MCC column</td>
<td>No quantification/identification of VOCs</td>
</tr>
<tr>
<td>Vacuum conditions</td>
<td>Ambient conditions</td>
<td>Ambient conditions</td>
<td>Ambient conditions</td>
</tr>
<tr>
<td>Slow</td>
<td>Fast, easy</td>
<td>Fast, easy</td>
<td>Fast, easy</td>
</tr>
<tr>
<td>Offline sampling</td>
<td>Offline sampling</td>
<td>Online sampling</td>
<td>Online sampling</td>
</tr>
<tr>
<td>Large, immovable set-up</td>
<td>Transportable</td>
<td>Transportable</td>
<td>Transportable</td>
</tr>
<tr>
<td>Very expensive</td>
<td>Cheap</td>
<td>Cheap</td>
<td>Expensive</td>
</tr>
<tr>
<td>Specific technician training</td>
<td>No specific operator training</td>
<td>No specific operator training</td>
<td>No specific operator training</td>
</tr>
</tbody>
</table>

Abbreviations: ppb, parts per billion by volume; ppm, parts per million by volume; ppt, parts per trillion by volume.

Properties of Breath Analyzing Techniques

An ideal breath analyzing method should be cheap, user friendly, noninvasive, and easily accessible without patient discomfort or traveling. The above described techniques developed for gaseous breath analysis, all have their specific advantages and disadvantages as summarized in Table 3.

Dogs, eNoses, and IMS have a high sensitivity and are transportable but do not identify the individual compounds. Offline sampling methods (like GC-MS or eNoses) use different bags and tubing material for breath sampling, are not in real time, and induce a risk of sample contamination which leads to potential biases for data comparison between research groups. The water content of breath samples is also pernicious for sensor analysis, as it causes sensor drift and leads to possible loss of information by dilution. The sampling technique must handle this by removing the water vapor before the analysis by preconcentration, increasing the risk of contamination, and losing some of the VOCs. When offline methods are used, mixed breath is stored in bags or tubes which can furthermore induce errors.

GC-MS analysis remains the gold standard, as it is very sensitive and allows VOC identification and quantification, albeit very expensive, time-consuming and laborious, and it requires a large vacuum-containing set-up and qualified technicians. Furthermore, in conventional mass spectrometers, the impact of electrons on polyatomic molecules and complex gas mixtures as breath induces large fragmentation of the molecules. This complicates the mass spectra interpretation and makes it practically impossible to carry out accurate quantitative analyses.

A more desirable technique is one in which the patient breathes directly (online) into the analytical equipment without any preconcentration steps and achieves the ionization of these gases with minimal fragmentation of the molecules which simplifies the outcome. This gives fast, real-time information about the breath composition, allows for instantaneous feedback, and decreases the chance of contamination. IMS coupled to an MCC has these features (Table 3) and subjects breathe directly into a CO2-controlled end-tidal breath collecting device collecting alveolar air in a sample loop followed by a fast analysis. A VOC database comprising correlated GC-MS with IMS spectra allows immediate VOC identification and the effective separation of humidity is an advantage.

Conclusion and Future Directions

Past and current asbestos consumption will lead to more cases of mesothelioma in the coming decade. Breath
analysis as a noninvasive diagnostic tool provides a fast, user-friendly, and cost-effective system which is not subjected to laboratory conditions. Hence, breathomics can become the missing link to screen for early cases in professional or environmental asbestos-exposed persons (i) to rule out diagnosis requiring a high NPV, (ii) to rule in diagnosis necessitating a high PPV and (iii) as a step-up diagnostic tool to enrich the fraction of screenees for further testing, requiring a low false-negative rate.

Although they are not tumor specific, one of the most abundant detected molecular groups in the breath of asbestos-exposed individuals are alkane representing the oxidative stress level (Table 2). Even though published studies were able to discriminate asbestos-related diseases from healthy controls, there is a lot of variation in sample size, study design, control groups, techniques, sampling procedures, the kind of sampled breath (mixed/alveolar), preconcentration techniques, and data-mining techniques. This makes it hard to compare the studies or draw clinically relevant conclusions at the present time.

Nevertheless, breath analysis will eventually become a useful tool for the diagnosis of asbestos-related diseases but the way to a validated and clinical implemented model is still harsh and cumbersome. A large, prospective, case–control study is to be performed using control groups with comparable characteristics that cover the possible confounding factors for asbestos-related diseases to strengthen the diagnostic tool’s discriminative power. Therefore, healthy, nonsmoking and smoking controls should be included as well as healthy asymptomatic asbestos-exposed individuals and participants with benign asbestos-related diseases. Lung cancer and mesothelioma patients should also be included, preferable treatment-naive and their VOCs should be analyzed by disease stage. Standardization of the sampling techniques is necessary to allow comparison with results obtained in the field. Models should be built on the basis of a training set and internally validated in a separate validation set accounting for ambient air, gender, smoking behavior, and age. External validation should be performed in an independent test set estimating the general error. Different data mining techniques (classification trees, discriminant analysis, support vector machines, random forests, principle component analysis) should be investigated in cooperation with (bio-)statisticians (75). To select appropriate VOCs representing MPM, comparisons with cell lines or xenografts and pre- and postoperative VOC analysis should be performed. However, the importance of identifying different compounds is debatable: from pathophysiological view, VOCs should be identified with MCC/IMS or GC-MS to link MPM-related VOCs to their specific pathogenesis, and hence allow investigating therapeutic targets. On the other hand, recognizing breath patterns with dogs or eNoses allows for a clinical diagnostic assessment and monitoring.

Finally, an international consortium, discussing and optimizing each technique in large-scaled validation studies is needed and therefore an International Association for Breath Research Task Force for breath sampling was recently founded. This could increase the speed of developing good diagnostic methods in asbestos-related diseases in a cost-effective way.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

Authors’ Contributions
Conception and design: K. Lamote, K. Nackaerts, J.P. van Meerbeeck
Development of methodology: K. Lamote, J.P. van Meerbeeck
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): K. Lamote
Writing, review, and/or revision of the manuscript: K. Lamote, K. Nackaerts, J.P. van Meerbeeck

Grant Support
This work was supported by a grant (STK 2010-205, STK 2012-223) from the Belgian Foundation Against Cancer (to K. Nackaerts and J.P. van Meerbeeck).

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received July 23, 2013; revised February 13, 2014; accepted March 11, 2014; published OnlineFirst April 4, 2014.

References
Breath Analysis in Asbestos-Related Diseases


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