CME review

Human exhaled breath analysis

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Objective: To review the fast-developing topic of assessment of exhaled breath components to improve the diagnosis and monitoring of respiratory and systemic diseases.

Data Sources: Review of the literature available in monographs and journals.

Study Selection: Articles and overviews on the broad spectrum of existing experimental and routinely applied methods to assess different aspects of human exhaled breath analysis were selected for presentation in this review.

Results: Exhaled breath constitutes more than 3,500 components, the bulk of which are volatile organic compounds in miniature quantities. Many of these characterize the functioning of the organism as a whole (systemic biomarkers), but some are related to processes taking place in the respiratory system and the airways in particular (lung biomarkers). Assessment of lung biomarkers has proven useful in airway inflammatory diseases. It involves direct measurement of gases such as nitric oxide and inflammatory indicators in exhaled breath condensate such as oxidative stress markers (eg, hydrogen peroxide and isoprostanes), nitric oxide derivatives (eg, nitrate and nitrates), arachidonic acid metabolites (eg, prostanoids, leukotrienes, and epoxides), adenosine, and cytokines. Integral approaches have also been suggested, such as exhaled breath temperature measurement and devices of the "electronic nose" type, which enable the capture of approaches have also been suggested, such as exhaled breath temperature measurementexhaled molecular fingerprints (breath prints). Technical factors related to standardization of the different techniques need to be resolved to reach the stage of routine applicability.

Conclusions: Examination of exhaled breath has the potential to change the existing routine approaches in human medicine. The rapidly developing new analytical and computer technologies along with novel, unorthodox ideas are prerequisites for future advances in this field.

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INTRODUCTION

Biomarkers are quantifiable indicators of physiologic function and disease activity that provide a practical basis for diagnosis and monitoring of pathologic states. They can be measured in different media belonging or emanating from an organism. Circulating blood is pooling together biochemical compounds and metabolites released from different tissues. A plethora of these are released in the ambient environment in the process of gas exchange taking place in the lung alveoli; other moieties are released from the airways to join the mix. More than 3,500 different components have been identified in exhaled breath, with the list continually growing. Several of them are gases, which constitute the bulk of the exhaled breath volume: nitrogen, oxygen, water, and carbon dioxide. Others are mostly volatile organic compounds (VOCs), and their concentration is miniature. Approximately 50% of the identified VOCs are of endogenous origin, and approximately 200 of these trace compounds are detected in average breath samples from the general human population.[1] Different sets of compounds account for the individual smell that characterizes a given subject, transient components can impart fluctuations due to the dietary regimen and other ambient influences, and still others can be associated with pathologic metabolisms specific to dif-

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ferent disease states. The complex matrix of exhaled breath components is referred to as *molecular breath signature.*

For many years physicians have been "sniffing" for specific odors in the air their patients exhale, hence the adjectives *uremic*, *hepatic*, and *acetone* found in medical texts to describe exhaled breath in the respective diseases. The limitations of this organoleptic approach were that diseases would be diagnosed at a very advanced stage when the systemic metabolism was totally compromised. Some 4 decades ago Linus Pauling demonstrated that minute amounts of multiple compounds could be detected and quantitatively measured by gas chromatographic analysis of exhaled air and urine vapors.[2] Increased levels of some of these compounds are indicative of systemic disorders and extrapulmonary organ failures, which could be referred to as *systemic biomarkers*. Since that time technological progress has made it possible to develop miniature instruments capable of measuring extremely large numbers of chemical analytes with limits of detection within the nanomolar and picomolar range.[3]

This marked improvement in the detective power of breath analysis has prompted the exploration of airway diseases by evaluation of biomarkers deriving from the airways and lung structures. These can be referred to as *lung biomarkers*. The rationale for this approach is based on research performed in the 1980s and early 1990s, when it was recognized that the substrate of asthma symptoms is inflammation of the mucosa and the underlying tissues lining the airways.[4] Once the immunologic mechanisms of this pathologic process set in motion, the process carries on with fluctuations despite treatment. Still further, while the vicious cycle of allergic inflammation spins, it brings about remodeling of the airways with early impairment and premature death.[5] These phenomena occur as a result of interplay of multiple cellular and fluid-phase ingredients. It was obvious that the invasive approaches, such as bronchoscopy with bronchoalveolar lavage, initially used to decipher the nature of asthma would not be applicable in routine clinical practice. The first noninvasive method that was validated to assess airway inflammation was the examination of induced sputum.[6,7] Although sputum analysis is still a method of reference, it could not establish itself as a routine standard because it is time-consuming, sophisticated, and expensive. It was tempting to look for biomarkers of inflammation directly in the exhaled air.

EXHALED BREATH ANALYSIS

Directly Determined Components of Exhaled Breath

Gases appearing in the exhaled breath as by-products of different metabolic pathways or after oral ingestion can be detected and quantitated. Thus, ethanol measurement has found wide application in the control of alcohol consumption by drivers. A recent application of interest is the installing of devices called Alcolocks or alcohol interlock systems in certain types of transportation vehicles in Sweden.[8] These are automatic control systems that are designed to prevent driving after excessive alcohol intake by requiring the driver to blow into an in-car breathalyzer before starting the ignition. The alcohol interlock can be set at different levels and limits.

Other interesting developments related to systemic biomarkers in exhaled breath are the attempts to directly assess gases associated with human morbidity, such as ammonia[9] and acetone,[10] with the latter being used also as a proxy for blood levels of glucose. The directly measured gases, which have been intensively investigated as biomarkers in lung morbidity, are nitric oxide (NO) and carbon monoxide.

Nitric Oxide

Gustafsson et al[11] were the first to detect NO, a highly reactive molecule, in the exhaled breath of animals and humans in 1991. Two years later, Alvin et al[12] reported an increase of NO in the exhaled breath of asthmatic patients, which gave an impetus to research aimed at assessing the usefulness of this and other gases in different clinical situations. As a result, a much better insight was gained about the role NO plays in the regulation of the smooth muscle tone of the pulmonary blood vessels and the bronchi. The increase in NO during inflammation has been demonstrated to be due to activation of inducible NO synthase expressed by epithelial cells in response to proinflammatory cytokines and oxidants.[13] Because expectations were high, several companies engineered devices using the principle of chemiluminescence to measure the minimal amounts of NO in the exhaled air (in the range of parts per billion), with which many dozens of studies were conducted, bringing the method to the stage of clinical use. The role of different extraneous factors, such as inspiration maneuvers, breath holding, exhalation flow rate, exhalation time, and oral pressure, with possible bearing on exhaled NO (eNO) measurement, has been elucidated.[14] Fractional eNO (FeNO) levels were shown to correlate with sputum eosinophil count, airway hyperresponsiveness, bronchodilator response, serum IgE levels, allergen skin prick testing, asthma symptoms, and lung function.[15,16] This led to the general belief that atopy is associated with higher levels of eNO. However, the analysis of different subgroups of atopic individuals now suggests that it is the active inflammation in atopic individuals with clinical manifestations of airway disease, rather than atopy itself, that accounts for the increased production of eNO.[14] Other patient characteristics, such as age, height, weight, body mass index, sex, and race, may affect the measurements to a lesser degree, as well as activities such as smoking, alcohol use, spirometric testing, sputum induction, and environmental exposures.[17,18] The currently available equipment and dedicated software allow accounting for different confounding factors.

These ground studies paved the way for standardization of the method, and subsequently the American Thoracic Society and the European Respiratory Society published recommendations for measuring FeNO, as it was officially denoted.[19,20] To increase the reproducibility, it was proposed that measurements were done in duplicate and a third measurement was to be made if the first two differed by more than 10%.

The present applications of NO measurement cover asthma diagnosis, monitoring, management, and treatment of asthma.[21,22] Thus, the sensitivity of FeNO measurement at levels higher than 20 ppb in patients with symptoms suggestive of asthma has been shown to be 88%, with a negative predictive value of 92%.[23] With the appearance of cheaper portable handheld devices based on electrochemical sensing on the market, the possibility was contemplated to perform serial measurements to monitor disease activity and to steer asthma control,[24] especially because it was demonstrated that the increase of FeNO precedes the decrease in peak expiratory flow rates.[25] Another lucrative possibility is to titrate the optimal maintenance dose of inhaled steroids.[26] Taken together, these applications held the promise to use FeNO to guide asthma management.[27] However, a carefully designed study by Szefler and coworkers[28] showed that the use of FeNO neither improved asthma control nor allowed reduction in the dose of inhaled glucocorticosteroid.[28]

An important advantage of the method is related to the noninvasive nature and the simplicity of the FeNO measurement, which makes it applicable in children and even infants.[29,30] The special promise in these youngest patients is held by the fact that there are virtually no objective measures to support the diagnosis and control of treatment in those younger than 5 years. Conclusive trials are still awaited to clearly demonstrate the benefit of FeNO measurements in the clinical setting.[31]

Carbon Monoxide

Another gaseous constituent of exhaled breath is exhaled carbon monoxide (eCO), which has been initially suggested as a measure of oxidative stress.[32] Its concentration in the exhaled breath derives from endogenous production by different tissues as a result of haem metabolism catalyzed by oxygenase enzymes.[33] It plays an important role as modulator of inflammation, cellular apoptosis, proliferation, and differentiation.[34] Carbon monoxide in the ambient air, which reflects air pollution from burning fossil fuels and tobacco smoking, influences its levels in the exhaled breath. It is measured with simple electrochemical methods by means of portable devices in the parts per million range. A recent meta-analysis assessing the value of eCO in asthma management concludes that eCO is elevated in asthmatic patients but that its levels only partially reflect disease severity and control.[35] Because of the confounding effect of tobacco smoking, eCO might be a potentially useful noninvasive biomarker of airway inflammation and oxidative stress in nonsmoking asthmatic patients. A specifically designed study challenges its utility as one time point measurement but still assumes some benefit in longitudinal follow-up.[36]

Indirect Measurements in Exhaled Breath Condensate

Most trace VOCs contained in exhaled breath currently cannot be directly measured because this requires the development of specific sensors with outstanding sensitivity and specificity. New opportunities have opened up by using exhaled breath condensate (EBC), obtained by cooling exhaled air, which contains water vapor and microdroplets. The composition of EBC is believed to reflect that of the airway epithelial lining fluid.[37] The microdroplets are the actual carriers of the multitude of VOCs, but little is known about their formation and detachment from the airway walls during breathing. They are diluted in the resulting EBC between 5,000 and 25,000 times.[38] The condensation of the water vapors in exhaled breath is achieved by exhalation through simple cooling devices, making EBC collection totally noninvasive and consequently particularly easy to perform even in children with severe disease.[39] A technique has been developed to safely obtain EBC even from infants using a face mask connected to a cooling device.[40] Influences confounding the accuracy of the measurements, such as smoking, alcohol consumption, equipment, exercise, mode and rate of breathing, nasal and salivary contamination, environmental temperature and humidity, and the nature of determination assays used, have been identified.[41,42] New techniques for diversifying the number of known biomarkers with increased detection threshold are already on the way.[43] Recommendations have been recently published on how to collect EBC in an attempt to minimize differences due to sampling devices and collection protocols.[44]

A large number of mediators have been measured in EBC, among which few have proven clinically useful. These can be grouped into biomarkers of oxidative stress, arachidonic acid metabolites, NO-related products, pH of the EBC, cytokines and chemokines, and proteins. A detailed overview of all the different components investigated in EBC has been recently published[45] (Table 1). Some of these EBC biomarkers correlate with asthma severity, lung function impairment, and airway remodeling, and others hold promise as identifiers of specific asthma phenotypes, such as aspirin-sensitive asthma and exercise-induced asthma.

Despite the encouraging positive results to date, the introduction of EBC in everyday clinical practice requires the resolution of some methodologic pitfalls, the standardization of EBC collection, and finally the identification of the reliable biomarkers that are reproducible, have normal values, and provide information about the underlying inflammatory process and the response to treatment. An important issue to be resolved is finding a reliable approach to account for the dilution of the microdroplets by the water vapor in the exhaled air. Three candidate markers of dilution (urea, total cations, and conductivity of vacuum-treated EBC) have been introduced, with the assumption that concentrations of each indicator in the epithelial lining fluid are similar to those in the plasma.[38] Accounting for the level of dilution of the EBC may help our judgment about the usability of certain markers of inflammation. Thus, measuring conductivity of vacuum-treated EBC samples to account for dilution resulted in a disappointing lack of differences between asthmatic patients and healthy controls for the potential candidates for airway inflammation biomarkers: adenosine and adenosine triphosphate.[46] The conclusion was that the relation between EBC

Table 1. Exhaled Breath Biomarkers Recognized as Meaningful in Asthma Diagnosis and Monitoring[a]

Oxidative stress biomarkers	Nitric oxide–related biomarkers	Arachidonic acid metabolites: eicosanoids	Others
Hydrogen peroxide	Nitrite/nitrate	Prostanoids: prostaglandins E_2, D_2, and $F_{2\alpha}$ and thromboxane B_2	pH
Isoprostanes (8-isoprostane, 15-$F_{12\text{-IsoP}}$)	Nitrotyrosine	Leukotriene B4 and cysteinyl leukotrienes C_4, D_4, and E_4	Purines: adenosine, adenosine triphosphate, adenosine monophosphate
Aldehydes and thiobarbituric acid–reactive products	S-Nitrosothiols	Epoxides	Cytokines: IL-2, -4, -5, -6, -8, -10, and -17, TNF-α, TGF-β, IFN-γ Chemokines: CCL-3, 4, 5 (RANTES), 11 (eotaxin),17, 22 Proteins: albumin, C-reactive protein, endothelin 1, others

Abbreviations: IFN-γ, interferon γ; IL, interleukin; TGF-β, transforming growth factor β; TNF-α, tumor necrosis factor α.
[a] Common features include highly reactive compounds and pronounced biological effects in minimal quantities, which were consistently different in asthma patients compared with healthy controls and patients with other lung diseases.

mediator concentration and EBC conductivity highlights the importance of further standardization of the EBC method and the need for more studies to understand airway droplet formation. Drawbacks of EBC analysis derive from the fact that the concentrations of mediators in EBC are low, nearing the detection limit of currently available techniques, so more sensitive methods, such as high-performance liquid chromatography, negative ion chemical ionization mass spectrometry, gas chromatography–mass spectrometry, nuclear magnetic resonance–based spectroscopy, and field asymmetric ion mobility spectrometry, should be considered for this assay.[43,45] Furthermore, the anatomical sources of the compounds measured in EBC are not well defined, and most of the measurements are not done in real time.

INTEGRAL ASSESSMENT OF AIRWAY INFLAMMATION BY MEANS OF EXHALED BREATH

Exhaled Breath Temperature

The deep structures of the lung typically have temperatures representative of the body core. The temperatures are determined by the blood flowing along the rich vascular network of the alveoli. The temperature of the inhaled air is tempered during its flow in and out of the branching airways, which have a separate system of blood supply. Because blood is the main carrier of thermal energy maintaining the core body temperature, processes that would modify its flow within the airway walls might reflect on the exhaled breath temperature. Several research teams have investigated the assumption that inflammation of the airways would influence the temperature of the air coming from the alveoli of the lungs and that this added signal could be picked by appropriate means.[47-49] In previous experiments it was demonstrated that in asthmatic adults and children there was correlation between the temperature of the exhaled breath and the bronchial blood flow, the FeNO levels, and the number of sputum eosinophils.[50,51]

Although the first experiments were made in sealed chambers with sophisticated equipment requiring extensive training of the patients to take the measurements, a specially designed, handheld instrument was built that allowed measurements to be taken in regular indoor environments without special requirements from the tested patients. It proved successful in differentiating asthmatic from nonasthmatic patients and picked the improvement in the condition of patients treated with inhaled anti-inflammatory drugs[52] and outlined the possibility of using this portable instrument for daily monitoring of airway correlating with parallel peak expiratory flow measurements.[53] Software improvements would make the device still friendlier to patients and physicians and would render the time for measurement minimal.[54]

Electronic Nose, Metabolomics, and Phenotyping

The VOC profiles can be assessed using integrative analysis by an electronic nose, a device that uses composite nanosensor arrays based on carbon black-polymer composite vapor detectors combined with inbuilt electronic learning algorithms, which can put together exhaled molecular fingerprints (breath prints). These recognition patterns can differentiate between different disease states.[55,56] Of particular interest are the studies demonstrating the ability of the electronic nose to differentiate between asthmatic patients and healthy controls and between asthma and chronic obstructive pulmonary disease patients.[57,58] A commercial device is now available, which is currently tested in different disease states. A different approach using DNA-coated sensors has also been reported.[59]

Metabolomics is another emerging field of integral assessment of exhaled breath components, so far using only EBC. The concept has been coined by Nicholson as systematic study of the unique chemical fingerprints that specific cellular processes leave behind, specifically of their small-molecule metabolite profiles.[60] This approach requires identification of a huge multiplicity of metabolitic

products characterizing different disease states. It has been proven applicable to EBC, but there is a way to go until it reaches clinical validity.[61]

The discussed integral approaches come in time with the advancement of electronic technologies, which make possible processing huge amounts of data. This could enhance the efforts to identify disease phenotypes, which parcel a disease entity into subgroups with common characteristics that may affect management. Thus, the common disorder asthma is probably a collection of different phenotypes rather than a single disease.[62] In the future, more specific biomarkers would certainly be needed to discriminate among different asthma phenotypes and guide individual treatments.

DISCUSSION

The present overview encompasses a broad range of techniques used to assess different aspects of human exhaled breath, some of which are at the proof-of-concept stage of development, whereas others have applied for research or clinical judgment. The more than 15-year-old saga of FeNO measurement is a typical example of the extensive route that needs to be covered. With more than 2,000 publications in PubMed on the topic, there are still controversies about its utility, reflected by the fact that the most recent Global Initiative for Asthma guidelines do not recommend its use for guiding treatment.[63] Recently, a well-defined track has been suggested in proving that a biomarker is apt for use in routine practice: the Standards for Reporting of Diagnostic Accuracy initiative.[64] These standards outline all particulars that need to be assembled until a specific biomarker reaches maturity: its definition and biological role, the characteristics of the study populations and the control groups in light of the target disease, technical details about the methods and equipment used, the data collection process and the statistical analysis, and the inferences about the clinical applicability.

CONCLUSION

Identifying biomarkers in exhaled breath, directly in the outflowing air or in EBC, is a lucrative approach that would help the diagnosis, monitoring, management, and treatment of systemic and lung diseases. There are 2 different but complementary trends: the creation of technology or the use of integral approaches to examine exhaled breath. Thus, the emphasis on asthma management will gradually shift from judgments made on the basis of subjective symptoms and objective lung function measurements to noninvasive evaluation of the type and level of airway inflammation.

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Objectives: After reading this article, participants should be able to demonstrate an increased understanding of their knowledge of allergy/asthma/immunology clinical treatment and how this new information can be applied to their own practices.

Participants: This program is designed for physicians who are involved in providing patient care and who wish to advance their current knowledge in the field of allergy/asthma/immunology.

Credits: ACAAI designates each Annals CME Review Article for a maximum of 1 credit toward the *AMA PRA Category 1 Credit™*. Each physician should claim only those credits that he/she actually spent in the activity. The American College of Allergy, Asthma and Immunology is accredited by the Accreditation Council for Continuing Medical Education to sponsor continuing medical education for physicians.

CME Examination

1-5, Popov TA. 2011;106:451–456.

CME Test Questions

1. Approximately, how many volatile organic compounds are detected in average breath samples from the general human population?
   a. 20
   b. 200
   c. 500
   d. 1,000
   e. 2,000
2. Which of the following activities affect fractional exhaled breath nitric oxide measurement?
   a. tobacco smoking
   b. spirometric testing
   c. sputum induction
   d. environmental exposures
   e. all of the above
3. What is exhaled breath condensate believed to reflect?
   a. the cellular content of the bronchial epithelial cells
   b. the salivary composition
   c. the blood serum
   d. the airway lining fluid
   e. the submucosal connective tissues
4. Isoprostanes are markers of:
   a. oxidative stress
   b. eosinophilic inflammation
   c. neutrophilic inflammation
   d. immune deficiency
   e. infection
5. What is the concept metabolomics based on?
   a. the measurement of a specific metabolite
   b. the assessment of multiplicity of small-molecule products left behind by specific cellular processes
   c. the evaluation of the body metabolism by measurement of gases released during breathing
   d. the assessment of the fluid phase of cell cultures
   e. the assessment of dietary regimens on the process of breathing

Answers on page 501.

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Answers to CME examination—*Annals of Allergy, Asthma & Immunology*, June 2011 Popov TA: Human exhaled breath analysis. *Ann Allergy Asthma Immunol*. 2011;106:451–456.

1. b
2. e
3. d
4. a
5. b