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Cardiovascular Biomarkers In Exhaled Breath

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Abstract

With each breath we exhale, thousands of molecules are expelled in our breath giving individuals a “breath-print” that can tell a lot about them and their state of health. Breath analysis is rapidly evolving as the new frontier in medical testing. The end of the 20th century and the beginning of the 21st century have arguably witnessed a revolution in our understanding of the constituents of exhaled breath and the development of the field of breath analysis and testing. Thanks to major breakthroughs in new technologies (infrared, electrochemical, chemiluminescence, and others) and the availability of mass spectrometers, the field of breath analysis has made considerable advances in the 21st century. Several methods are now in clinical use or nearly ready to enter that arena.

Breath analysis has the potential to offer relatively inexpensive, rapid, noninvasive methods for detecting and/or monitoring a variety of diseases. Breath analysis also has applications in fields beyond medicine, including environmental monitoring, security and others. This review will focus on exhaled breath as a potential source of biomarkers for medical applications with specific attention to applications (and potential applications) in cardiovascular disease.

INTRODUCTION AND BACKGROUND

Our exhaled breath is a complex matrix with thousands of molecules that constitute a “breath-print” that carries information about us (similar to a fingerprint) and certain information about our state of health (similar to our blood or urine). One can reasonably argue that the history of using breath as a biomarker is as old as medicine itself. Hippocrates described fetor oris and fetor hepaticus in his treatise on breath aroma and disease [1]. In the modern era, clinicians frequently notice that patients with certain medical conditions like diabetes, liver cirrhosis, or kidney failure have distinct odors to their breath. Active research

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in this area is uncovering the scientific and chemical basis for these clinical observations. With modern mass spectrometry (MS) instruments, scientists are now able to identify thousands of unique substances in exhaled breath [2]. In addition to carbon dioxide and oxygen, exhaled breath includes several other elemental gases like nitric oxide (NO). Our exhaled breath also contains a large number of volatile organic compounds (VOCs). Some of these compounds have been identified and linked to disease states but the vast majority are still under investigation. Exhaled breath also carries aerosolized droplets collected as "exhaled breath condensates" [3] and particles [4] that contain non-volatile compounds such as proteins as well.

The field of breath analysis is rapidly evolving as the new frontier in medical testing for disease states in the lung and beyond [1]. Breath analysis is now used to diagnose and monitor asthma, hemolysis, lung and other cancers, and heart transplant rejection among other applications [5–9]. Major breakthroughs in MS and sensor technologies have led to considerable advances in breath analysis in the last few years. Several breath biomarkers are now in clinical use and many more are being studied and tested for that purpose. Breath analysis has the potential to offer relatively inexpensive, rapid, noninvasive methods for detecting a variety of diseases. Breath analysis also has applications in fields beyond medicine, including environmental monitoring, security and others. This review will focus on exhaled breath as a potential source of biomarkers for medical applications with specific attention to applications (and potential applications) in cardiovascular disease.

There are several potential advantages for breath analysis as a medical test (Table 1). The method is non-invasive (the sample is relatively easy and painless to acquire), the sample is likely to be rich with information (a single test can scan for signatures of many abnormalities or markers of disease), and has the potential for low-cost and lends itself to easy administration. Due to non-invasive nature and ease of administration, breath analysis may also be used in repeated testing to track the response to therapy.

Exhaled nitric oxide is generally considered the example to follow when it comes to the development of breath tests for clinical use. The process is well described in the literature from discovery [10], to understanding of the biology [11–13], to standardization of methods [14–15], to realizing the strengths [16–17] and limitations [18], to approval by the regulatory agencies [19], to acceptance in clinical application and practice [20].

EXHALED NITRIC OXIDE (F_ENO)

NO has long been known as an atmospheric pollutant present in vehicle exhaust emissions and cigarette smoke, but the discovery that it is a biological mediator led to many breakthroughs in our understanding of human physiology and disease [21–22]. NO is endogenously synthesized by one of three nitric oxide synthases (NOSs) which convert L-arginine to L-citrulline and NO in the presence of oxygen and several cofactors. All three NOSs (type I, II and III) are widely expressed in various tissues including the lungs, the heart and the pulmonary and systemic circulations [21, 23–24].

The advent of chemiluminescence analyzers in the early 1990s allowed the detection of low parts per billion levels of NO in exhaled breath [10]. The first obvious clinical application was

in patients with asthma who were found to have high levels of NO in their exhaled breath[25] that decreased in response to treatment with corticosteroids [26]. This quickly prompted the evaluation and later the adoption of exhaled NO as a non-invasive method to diagnose asthma and monitor the response to anti-inflammatory therapy. Potential advantages for exhaled NO include its non-invasive nature, ease of repeat measurements, and use in children and patients with severe airflow obstruction made it a potentially attractive test in this patient population [27]. Improvements in our understanding of inflammation in asthma coupled with advances in technology and standardization of the methodology made FE_{NO} measurement simple and allowed for its evaluation in different settings from diagnosis, to monitoring, to screening, and possibly others. FE_{NO} is approved by the FDA for monitoring of airway inflammation in patients with asthma and is currently in clinical use for this purpose [20].

F_ENO In Pulmonary Hypertension

NO is one of the important pathophysiologic mediators of pulmonary hypertension [28–29]. In addition to vasodilation, NO regulates endothelial cell proliferation and angiogenesis, and maintains overall vascular health [24]. Interestingly, patients with pulmonary arterial hypertension (PAH) have low F_ENO values [24]. Individuals with PAH also have lower than normal concentration of NO reaction products in the bronchoalveolar lavage fluid which is inversely related to the degree of pulmonary hypertension [30]. Although this is a far more complex issue than the simple lack of a vasodilator [31], replacement of NO seems to work well in treating the problem [29]. Therapies that target the NO pathway have revolutionized the treatment of this disease, including the widely-used phosphodiesterase type 5 (PDE5) inhibitors, which prevent the breakdown of the NO effector molecule 3', 5'-cyclic guanosine monophosphase (cGMP), thus prolonging NO effects on tissues [31–32]. The NO deficiency state in patients with PAH also improves with other therapies that do not directly target the NO pathway like prostacyclins and endothelin receptor antagonists [33]. This seems to also have a prognostic significance, with improved survival in patients who respond to therapy with a higher F_ENO level compared to those who do not change their NO levels in response to therapy [18]. The low F_ENO levels in patients with PH and the improvement with effective therapies suggest it may be a promising biomarker for this disease. More studies are needed to determine whether serial monitoring of F_ENO levels may be a useful noninvasive marker to evaluate response to or failure of medical therapy in these patients [34].

F_ENO and exhaled CO In Heart Failure

Nitric oxide has been studied extensively in cardiovascular disease since it is involved in the regulation of diverse physiologic processes including vasorelaxation and platelet inhibition [35]. Heart failure is associated with abnormalities in vasoregulation as a result of endothelial dysfunction. Increased NO in heart failure is presumably to counter vasoconstrictive forces, and is correlated with severity of heart failure. Plasma nitrate, an index of whole body endogenous NO production, is significantly increased in patients with heart failure [3637–38]. Significant dysregulation of endogenous nitric oxide production has been shown in patients with advanced ischemic or non-ischemic cardiomyopathy when compared to control subjects [39].

Exhaled NO did not differ between cardiomyopathy and controls at rest. Post exercise, however, there was a significant increase in exhaled NO in cardiomyopathy patients, but the levels were maintained in normal subjects. Interestingly, while, not as potent of a vasodilator as NO, dysregulation in CO and its levels in exhaled breath has also been implicated in heart disease [35]. In the same study mentioned above patients with cardiomyopathy had lower resting and post exercise exhaled CO compared to controls. Following exercise, a significant decrease in exhaled CO was observed in cardiomyopathy patients [39]. CO is produced endogenously by the action of heme-oxygenase that converts heme substrate to CO [40]. The physiologic effects of CO are less well understood than NO. In the cardiovascular system, CO has been shown to cause vasodilation and suppression of the hypertensive response and reduce neointimal development following arterial injury [41–4344]. Furthermore, CO suppresses pro-inflammatory cytokines and ameliorates postischemic myocardial dysfunction [45–46]. It has also been shown to inhibit rejection following cardiac transplants in rodents [47], prevent endothelial cell apoptosis [48] and inhibit platelet aggregation [49].

The discrepancy in the regulation between these two vasoregulatory molecules based on exhaled breath of patients with cardiomyopathy may be related to difference in sources. Exhaled CO arises predominantly from systemic production and is released to the alveolar space whereas exhaled NO is predominantly from the airway [50]. This is consistent with findings that exhaled NO is not reflective of systemic NO production [51–52]. In addition, some reports suggest negative modulation and cross-inhibition between the two molecules [40, 53] which may help shed a light on the pathobiology of heart failure and possibly provide simple non-invasive methods to monitor the disease.

EXHALED VOLATILE ORGANIC COMPOUNDS (VOCs)

Many VOCs are present in exhaled breath which can reflect the current physiologic state of an individual. Changed levels of VOCs in diseased patients, compared to healthy individuals, can provide insight into abnormal metabolism. Although there are many VOCs present in exhaled breath, those reflecting known metabolic pathways and physiologic processes provide tremendous possibilities in diagnostics and disease management. A selected list of VOCs implicated in disease processes are outlined in Table 2. The more relevant ones for cardiovascular disease will be discussed in more detail in the following sections.

VOCs of Cholesterol Metabolism

Several VOCs can be linked directly to cholesterol metabolism in the body, with breath isoprene being the signature molecule. Isoprene production and degradation occur in humans as a byproduct of cholesterol synthesis (Figure 1) and changes in its level can be measured in exhaled breath [54]. Breath isoprene levels can be decreased in humans by administration of HMGCoA reductase inhibitors, which block the enzyme responsible for the production of mevalonic acid (HMGCoA) during cholesterol biosynthesis (Figure 1) [54]. By measuring exhaled breath isoprene in patients on lipid lowering therapies, exhaled breath analysis can potentially provide an easy, non-invasive method for monitoring the effectiveness of treatment. While this is a very promising application for breath analsysis, several factors may contribute to exhaled breath isoprene and they need to be considered and

the sampling and measurement methods need to be standardized before use in a clinical setting. Breath isoprene is age dependent, and males typically have higher breath isoprene levels than females [55]. In addition, a circadian rhythm has been suggested to occur with breath isoprene with levels peaking between 2am and 6am [54].

VOCs in Oxidative Stress

Oxidative stress, or lipid peroxidation, is a term referring to the generation of reactive oxygen species (ROS) that catalyze the breakdown of polyunsaturated lipids into lipids with a carbon radical. The carbon radical then becomes a lipid peroxy radical by combining with molecular oxygen which can go on to breakdown other polyunsaturated lipids. This type of oxidative stress has been shown to occur in heart transplant rejection, acute myocardial infarction (MI), and several respiratory diseases [54]. Exhaled breath pentane, a by-product of lipid peroxidation, increases shortly after tissue injury [54]. Exhaled breath ethane is produced in a similar mechanism as pentane, and the two may be coupled together to generate a noninvasive test for tissue injury following acute myocardial infarction or other cardiovascular injury [54].

VOCs From Gut Bacteria

Gut bacteria are increasingly being implicated in the pathobiology of several diseases and health conditions including cardiovascular disease [56]. Metabolism by intestinal gut flora can also contribute to the many different types of VOCs expelled with each breath. Several of the compounds have been implicated in disease processes such as atherosclerotic plaque formation, non-alcoholic steatohepatitis (NASH), and obesity [54, 56]. The ability to monitor these compounds in exhaled breath could provide clinicians with an easily detectable, non-invasive method for disease management and treatment efficacy.

Exhaled breath ethanol has been a test for alcohol intoxication for many years, however small amounts of exhaled ethanol can be measured in individuals who abstain from alcohol. The endogenously produced ethanol is generated as a metabolic byproduct of bacterial metabolism [54]. It has been related to obesity in rats, and was shown to decrease following administration of a poorly absorbed antibiotic to remove the gut bacteria [54]. The measurement of endogenously produced ethanol may have diagnostic value in monitoring the development of NASH in obese individuals who abstain from alcohol consumption.

Acetaldehyde can be detected in small concentrations in exhaled breath, often resulting from the oxidation of ethanol. The hypothesis has been made that individuals with increased concentrations of acetaldehyde may have low-activity aldehyde dehydrogenase enzymes, which would limit the amount of ethanol that could be converted to acetaldehyde [54].

Methane, another compound produced from gut flora, is an indicator of carbohydrate malabsorption [54]. More specifically, when lactose and other sugars are poorly absorbed in the small intestine, they pass into the colon where bacteria metabolize them and produce methane (and often hydrogen) as metabolic byproducts.

Other VOCs

Acetone is produced in the liver following the degradation of acetyl CoA. When the body utilizes fat rather than glucose for energy such as during dieting, fasting, or starvation, acetone production increases. Elevated levels of acetone can be measured in exhaled breath, and has been demonstrated in the exhaled breath of patients with diabetes mellitus.

Endogenous reduction of acetone leads to the formation of 2-propanol. 2-propanol is a VOC that can be measured in the exhaled breath at concentrations lower than acetone. However, the actual concentration of 2-propanol that is generated from acetone may be difficult to determine accurately due to its use as a disinfectant in many hospital settings.

Sources of exhaled breath compounds need not be limited to human physiology. As stated previously, bacteria in the gut and mouth can often be sources of many volatile compounds in the breath. Hydrogen sulfide is a by-product of bacterial metabolism in the mouth, and can be a sign of periodontal disease [54]. Elevated levels of the compound have been observed in exhaled breath, and these levels can change significantly following a simple mouth rinse.

In addition to VOCs produced within the body, exogenous sources of compounds need to be considered as well. Many compounds measured in exhaled breath occur following ingestion of certain foods and beverages (ethanol or NO), or inhalation of car exhaust and cigarette smoke. Benzene and acrylonitrile are two VOCs that are present in elevated concentrations in individuals exposed to tobacco smoke. The large majority of both compounds are conjugated by the cytochrome P450 enzymes in the liver and excreted in the urine; however, the rest is exhaled in the breath unchanged. The ability to monitor the smoking habits or exposure of patients could provide evidence that lifestyle changes must occur to limit their exposure to cigarette smoke.

RELEVANT TECHNOLOGY

There are many existing technologies that can be tailored for exhaled breath analysis. The techniques that prove most useful from a clinical standpoint are those that can provide real-time feedback in an accurate and reproducible manner. Table 3 provides a selected list of breath tests currently approved by the FDA for clinical use. In this section we will discuss some of the current and upcoming technologies that are applicable to breath testing.

Mass Spectrometry

Gas chromatography(GC) with MS is an effective method for measuring trace compounds in exhaled breath [57]. Mass spectrometry has proven to be a useful tool in the discovery of volatile compounds in exhaled breath. GC-MS is the most common form of measuring volatiles. However, due to the large volume of gas necessary for analysis and the requirement of a pre-concentration system for analysis, this method may be impractical for real-time monitoring of patients. Additionally, calibration with known gases is needed prior to analysis of gaseous compounds. In order for these technologies to move forward into clinical practice, they will need to become more practical through online sampling methods and smaller instrument size to accommodate the limited space available in hospital settings. Due to these limitations of GC MS, several technologies have emerged that provide certain

advantages for application in breath analysis. A comparison of the advantages and disadvantages of selected technologies suitable for breath analysis (including GC MS) is provided in Table 4.

Proton transfer reaction (PTR)-MS is a fast, accurate method for analyzing exhaled volatile compounds. This system utilizes a precursor ion (H_3O+) to transfer a proton to gaseous compounds introduced to the precursor within the system. The protonated volatile compounds can then be quantified and reported as concentrations at the parts per billion level [57]. This method has the advantage of producing real time feedback of volatiles present in the exhaled breath. The use of a single precursor ion, H_3O+ may limit the detection of the wide range of volatile organic compounds as not all volatiles will react with H_3O+ to produce a full range of reaction products. The fact that this method provides exhaled breath data as a mass-to-charge ratio also makes it difficult to identify specific VOCs. In order to identify a peak of interest, alternative techniques such as GC-MS would be required.

Identification of VOCs of interest is necessary in determining the metabolic pathways involved in their production. A diagnostic breath test would have a much stronger rationale when the metabolic origin of a VOC implicated in disease is identified and the reason for the increased VOC production is linked to a specific pathway associated with the disease of interest.

Selected ion flow tube (SIFT)-MS is a method similar to PTR-MS, in that precursor ions react with incoming gas compounds to generate predictable product ions which are then quantified. This method is fast, with the ability to collect online samples for real-time feedback of volatile compounds in the exhaled breath, and accurate down to the parts per trillion levels for several VOCs [57]. An advantage that SIFT-MS has over PTR-MS is the use of three precursor ions rather than one to react with incoming gas compounds. This method allows for a broader range of volatiles to be measured compared to PTR-MS due to the different reactions that can occur with the three precursor ions. However, this method is subject to similar limitations as PTR-MS, in that positive identification of specific volatiles is impossible since results are reported as a mass-to-charge ratio. Other methods such as GC-MS would be necessary to correctly identify a compound of interest.

Sensor Technology

An ideal device for clinical breath analysis needs to be compact, portable, room-temperature operable, user-friendly, highly sensitive, highly selective, robust, precise, accurate, capable of real-time measurement, and inexpensive. While the different MS technologies techniques discussed above have been well-suited and widely used for the discovery phase, they have several drawbacks which reduce the likelihood their use for point of care testing [58–59]. So, while MS will continue to have an important role in biomarker discovery, its use in clinical settings will continue to be limited, even if the instrument is capable of real-time analysis. Breath analysis, even at the discovery phase, needs to move towards online sampling, which eliminates many of the confounding variables introduced by breath collection, concentration, and storage. But this transition is unlikely to happen without novel, highly portable sensing systems that require more compact and portable sensor systems. Electrochemical and optical sensors are widely used to measure elemental gases like oxygen

and carbon dioxide and more recently NO. These sensors, however, may not be well suited for measuring the larger volatile organic compounds.

Quantum cascade lasers (QCLs) have seen an increase in interest due to new methods in their production that allow for high selectivity and sensitivity of trace volatile organic compounds [54]. The ability to develop small, robust sensors for analysis of exhaled breath compounds makes QCLs a promising method for exhaled breath analysis. These devices can be made to detect wavelengths over the wide range of 3 to 20 μm [54]. Important technical parameters for QCLs fabricated for use in breath analysis include high optical power, single frequency operation, good spectral purity, and wide wavelength tunability [54].

Electronic Nose / Sensor Arrays

Similar to the human (or animal) nose, an electronic nose can identify a “smell-print” that it is trained to recognize. Unlike MS technology (and similar to their human counterparts) these devices cannot identify specific compounds that are responsible for a particular smell or smell-print. The electronic nose detects patterns in complex mixtures of volatile organic compounds in breath via an array of nanosensors. The nanosensors undergo a reversible reaction when exposed to compounds in the exhaled breath [60]. Furthermore, the resistance of each sensor is unique to the reactant compound; thus, a pattern (or “smell-print”) can be generated that is unique to a specific individual or possibly a specific disease [60]. A major limitation for the electronic nose and pattern recognition for use in clinical breath analysis is the inability to identify the volatile compounds they react to. So while the changes to the sensor array in response to breath from and idividual with a certain disease may be unique and reproducible, it is practically impossible to link the smell-print (or the VOCs that generate it) to the underlying pathobiologic process of the disease under consideration. This puts significant limits on the clinical and practical use of these devices.

Smart Sensor Systems

The limitations of the electronic nose led to growing interest in a different type of sensor array known as the “orthogonal” electronic nose. These devices are made up of an array of sensors just like the “traditional” electron nose but in this case each component sensor is designed to detect and measure a specific compound in the breath. With the advancement in the miniaturization in sensor technology, it is becoming more and more feasible to build these sensors into compact and portable devices. A *Smart Sensor System* is, at a minimum, the combination of a sensing element with processing capabilities provided by a microprocessor[61]. A more expansive view of a Smart Sensor System is a complete self-contained sensor system that includes the capabilities for data storage and processing, self-contained power, and an ability to transmit or display informative data to a user, operational system, or monitor station (e.g. a clinical data center). Recent technical advances in this area combine this Smart Sensor System approach with microsensor platform technology[62–65] produced using Micro Electro Mechanical Systems (MEMS) technology. The advent of MEMS technology allows the production of sensors of decreased size, weight, and low power consumption. Batch processing is used to produce many of the same type of sensor in one fabrication run with precise control of sensor structure, while at the same time reducing costs of production. New sensor platforms provide very different types of information and

these sensors are meant to have limited cross-sensitivity (i.e., be orthogonal in their response)[62]. Integration of the information from these orthogonal sensors has been shown to provide detailed information in environmental applications [64–65] that can be easily extended to breath analysis [66–67].

BREATH COLLECTION AND SAMPLING METHODS

There are several different methods for collection of exhaled breath for testing. These collection methods vary depending on the instrumentation and technology used.

Online Sample collection

In this method individuals exhale directly into the instrument that analyzes the breath. Instruments that utilize online sampling methods have potential to provide real-time feedback of volatile organic compounds present in exhaled breath. Technologies such as chemiluminescense, PTR-MS, and SIFT-MS utilize online sampling to generate rapid results from exhaled breath. Future sensors and QCLs designed for exhaled breath analysis will focus on this online method of sample collection.

Offline sample collection

Several instruments, most notably the mass spectrometers, are limited by their size, and thus portability. When online sampling is not possible due to transport of the instrument to the patient or vice versa, offline sampling is usually used. Recent advances in offline sampling methods have employed the use of Mylar or Tedlar bags to capture an individual's exhaled breath sample [15]. The bags must then be fitted with a sampling port to allow the instrument of choice to sample the breath from the bag. Disadvantages of this method include compounds being lost by absorption onto the surface of the bag, as well as volatiles being contributed by the bag itself.

Mixed expiratory sampling

Mixed expiratory samples contain air that participates in gas exchange in the alveoli of the lungs, as well as gas contained in the upper airway. This method can make a breath sample very complex since endogenous sources of VOCs intermix with exogenous compounds found in the ambient air [49]. Further complicating a mixed expiratory sample are airway sources of volatile compounds such as NO. This is the simplest way to sample a breath but it does not allow the determination of the origin of VOCs in the analyzed breath sample.

End-tidal sampling

End-tidal sampling involves collection of air that is closest to the alveolar-capillary interface in the lung, theoretically providing important metabolic information from the blood [68]. By observing and targeting specific portions of an exhaled breath sample, one can essentially trap and analyze different parts of the breath [68]. By utilizing the differences between exhaled and ambient levels of CO_2, one can accurately partition different portions of exhaled breath, such as alveolar air [68]. This can prove to be versatile if one wishes to study compounds released from the airways such as NO, or compounds from the alveolar air.

Exhaled breath condensate

Aerosolized droplets in exhaled breath can be captured by a variety of methods and analyzed for a wide range of biomarkers from metabolic end products to proteins to a variety of cytokines and chemokines and the possibilities continue to expand [69]. A major hurdle that faced this field as it transitions from the laboratory to clinical testing has been the standardization of sample collection methods[3, 69].

FUTURE DIRECTIONS

It is likely that the spectrum of breath analysis applications (medical, environmental, intelligence, etc) will continue to expand. This will require standardization of sample collection, the use of novel methods (to present, analyze, and report data), intellectual property issues (including funding and commercialization), and interaction with regulatory agencies. Progress will likely require getting early input from potential end-users (regarding needs and expectations), and addressing the critical need to study and understand the biologic relevance of the compounds present in exhaled breath. To advance the clinical applications of breath analysis, there has to be a close collaboration between technical experts who typically have a device looking for clinical indication, the medical experts who have the clinical problem looking for a test/biomarker that can be helpful in diagnosis or monitoring, and industry/commercial experts who can build and commercialize the final product.

SUMMARY / CONCLUSION

Our exhaled breath contains a vast array of substances and molecules that hold great promise for monitoring our health and for the diagnosis and management of a variety of clinical` conditions including cardiovascular diseases [70]. With recent advances in technology, essentially anything in the blood that is potentially volatile or has a volatile metabolite can be measured in exhaled breath [71]. This includes substances we produce endogenously as part of our normal (or disease-related) metabolism whether this is local in the lung or systemic in origin [3, 18, 72–73]. Since we are constantly inhaling air from our environment as we breathe in the ambient air, exhaled breath can also reflect our environmental exposure(s) [74]. Furthermore, our breath contains volatile compounds produced by our “internal environment”: the bacteria in our gut and mouth [72]. Add to all of those volatile byproducts generated from our diet, medications, drugs, or toxins that we are exposed to and you get a very rich matrix that has great potential to revolutionize and personalize medicine [75]. Tackling such a monumental task requires transdisciplinary collaborations and partnerships among all the stakeholders and any and all other disciplines that can inform the field. This includes but is not limited to medical professionals (physicians, researchers, biologists, etc.), scientists (chemists, physicists, biochemists, statisticians, etc.), engineers, commercial and industrial partners, and regulatory agencies, among others [75].

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Abbreviations

CO	Carbon monoxide
FENO	Fraction of exhaled NO
GC	Gas chromatography
MI	Myocardial infarction
MS	Mass spectrometry
NO	nitric oxide
NOSs	Nitric oxide synthases
PAH	Pulmonary arterial hypertension
PTR	Proton transfer reaction
QCL	Quantum cascade laser
SIFT	Selected ion flow tube
TMA	Trimethyl amine
TMAO	Trimethyl amine N-oxide
VOCs	Volatile organic compounds

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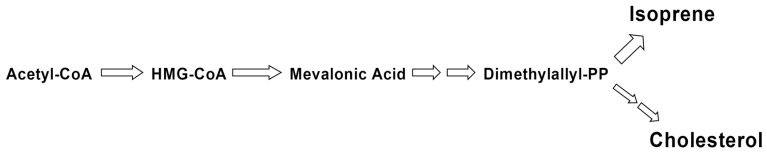

Figure 1.

A simplified schematic of the metabolic pathway of cholesterol synthesis. The process leads to the formation of the volatile organic compound isoprene which can be measured in exhaled breath.

Production of the VOC isoprene during cholesterol biosynthesis

Table 1

Potential advantages and limitations of exhaled breath analysis

Advantages	Limitations
Non-invasive and non-intrusive	Confounders such as diet and environment
Allows for repeated measurements	Lack of standardization
Can be inexpensive	Poor reliability of prototypes
Potential for portability	Storage
Potential for real-time results	Physician acceptance
Personalized medicine (“breath-print”)	

Table 2

Selected VOCs in human breath relevant to physiology and disease

Compound	Potential source	Implications for disease	Technology
2-propanol	Endogenous reduction of acetone/exogenous sources	Various diseases	GC–MS, SIFT–MS, SPME GC–MS
Acetaldehyde	Ethanol oxidation/gut flora	Low activity enzyme polymorphism	GC–MS, SIFT-MS
Acetone	Acetyl-CoA metabolism	Diabetes mellitus	GC–MS, SIFT–MS laser spectroscopy, SPME GC–MS
Acrylonitrile	Exogenous/tobacco smoke	Smoke exposure	GC-MS, SIFT-MS
Benzene	Exogenous/tobacco smoke/automobile exhaust	Lung and breast cancer/smoke exposure	GC–MS, SIFT–MS
Ethane	Lipid peroxidation/oxidative stress	Various diseases	GC–MS, MIR, laser absorption spectroscopy
Ethanol	Bacterial metabolism	NASH, obesity	SIFT–MS GC–MS, SPME GC–MS
Hydrogen sulfide	Oral bacteria	Periodontal disease	Gas chromatography, SIFT-MS
Isoprene	Cholesterol synthesis	CVD	GC–MS, SIFT–MS
Methane	Bacterial metabolism	Carbohydrate malabsorption	GC–MS, laser spectroscopy, mid infrared technology, methane breath test
Nitric oxide	Airway inflammation	Asthma/allergy/PH	MIR, chemiluminescence analyzer, laser-multi pass cell, laser cavity enhanced technique, NO analyzer
Pentane	Lipid peroxidation/oxidative stress	Various diseases	GC–MS, laser spectroscopy, SIFT–MS
TMA	Gut flora	Atherosclerosis, CVD	Gas chromatography, SIFT-MS

Abbreviations:

CVD: Cardiovascular disease

GC-MS: Gas chromatography-mass spectrometry

NASH: Non-alcoholic steatohepatitis

PH: Pulmonary hypertension

SIFT-MS: Selected ion flow tube-mass spectrometry

SPME-GC/MS: Solid-phase micro extraction- gas chromatography/mass spectrometry

Table 3

Selected breath analysis tests currently approved by the FDA

Molecules of interest	Indication	Product Name	Technique	Manufacturer	FDA approval date
$^{13}CO_2/^{12}CO_2$	*H. pylori* infection	BreathTek UBT for H. pylori Kit (BreathTek UBT Kit) and Pediatric Urea Hydrolysis Rate Calculation Application (pUHR-CA), Version 1.0	Infrared spectrophotometry	Otsuka America Pharmaceutical, Inc. (OAPI)	February 22, 2012
NO	Asthma and airway inflammation	NIOX MINO	Electrochemical sensor	Aerocrine	March 3, 2008
CO	Carbon monoxide poisoning	ToxCO	Electrochemical sensor	Bedfont Scientific Ltd	February 21,2008
H_2	Lactose malabsorption	Micro H_2 Breath Monitoring Device with Hydra Software Utility	Electrochemical sensor	Micro Medical Ltd	May 19, 2004
Alkanes (C4–C20)	Grade 3 Heart Allograft Rejection	Heartsbreath	GC-MS	Menssana Research, Inc.	February 24,2004
NO	Asthma and airway inflammation	NIOX^R	Chemiluminescense	Aerocrine	April 30, 2003

Abbreviations:

CO: Carbon monoxide

CO_2: Carbon dioxide

GC/MS: Gas chromatography/mass spectrometry

H_2: Hydrogen

NO: Nitric oxide

Table 4

Selected technologies used in breath analysis

Technology	Detection limit	Advantages	Disadvantages
GC/MS	ppt levels	Highly selective and sensitive	Long sampling time/need for standards/pre-concentration required
PTR-MS	Low ppb levels	Real-time analysis	Narrow range of detectable compounds/ impossible to identify compounds
SIFT-MS	Low ppb levels/ppt levels	Real-time analysis/broad range of detection	Impossible to identify compounds
QCL/Mid-IR	Low ppb levels	Real time analysis/potential for portability and miniaturization	Currently limited by available technology to reach sufficient specificity/selectivity required for practical use
Sensor Array / Electronic Nose	N/A	Real-time analysis/potential for portability and miniaturization	Pattern recognition makes identification of compounds impossible

Abbreviations:

GC/MS: Gas chromatography/mass spectrometry

ppb: parts per billion

ppt: parts per trillion

PTR-MS: Proton transfer reaction- mass spectrometry

SIFT-MS: Selected ion flow tube- mass spectrometry

QCL/Mid-IR: Quantum cascade laser/mid-infrared