Exhaled Breath Analysis

A Review of Clinical Applications to Determine Oxidative Stress

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Medical diagnostics are powerful tools that help clinicians distinguish health from various disease states, and even have the potential to predict future outcomes. Breath assessment has been used since classical Greek times to assist in medical diagnosis, but recently has benefited from major technological advances in chemical assay methods. Every breath provides a signature "breathprint" which is comprised of a unique combination of thousands of molecules. This signature can provide information that relates to the individual patient's health or disease states. Hippocrates was the first to describe *fetor oris* and *fetor hepaticus* in his dissertation on the aroma of breath and its relation to disease [1]. In the mid 1800's, Nebelthau determined that breath acetone is present in the breath of diabetic patients [2]. In 1874, Anstie isolated ethanol from breath [3]. And in the last few years, measurement of breath aldehydes has opened up a powerful window into understanding oxidative stress.

Technical advances throughout the 20th century and into the 21st century enabled the recognition of the vast quantities of information contained in human breath. In the 1970's Linus Pauling, using gas based chromatography analysis, determined that breath contained over 250 constituents. With the advent of more sensitive tools such as mass spectrometry and gas chromatography, and by combining the two techniques, we now know that there are well over 1000 individual components in exhaled breath. These substances are present as molecular gases, volatile organic compounds and aerosolized droplets collected as 'exhaled breath condensate' that contain both volatile and non-volatile organic compounds.

These categories form the basis for the two media used to study components in exhaled breath - exhaled breath and exhaled breath condensate. Exhaled breath is a non-condensing method that captures vapor phase molecules, whereas exhaled breath condensate is a newly considered body fluid collected by cooling breath and analyzing dissolved components within the aqueous matrix.

Transitioning from the laboratory to the clinical arena is a relatively new development. Over the last decade, there have been significant technical advances allowing new devices to be developed. Several products have either entered or are about to enter routine clinical use. Commercially available analyzers can measure Nitric Oxide ("NO") levels in exhaled breath to the parts per billion [ppb] and carbon monoxide levels to the parts per million [ppm] range [4]. Sensitive mass spectrometers and gas chromatographs can quantify volatile compounds on breath down to the parts per trillion [ppt] range. This technique can also be used to analyze aerosolized droplets in exhaled breath for a variety of biomarkers and the possibilities continue to expand [5].

Exhaled permanent gases

NO, a free radical, is a gaseous signaling molecule which plays a critical role in normal airways and in blood vessel tone regulation. NO is produced by NO synthase in the normal human airway. Normal exhaled breath contains low PPB levels of NO, however, asthmatic patients, who have increased expression of inducible NO synthase in their airways[6] prove to have an elevated concentration of FeNO [7]. Anti-inflammatory medications like inhaled corticosteroids cause a reduced expression of inducible NO synthase [8] and also cause a decrease in FeNO levels.

Levels of exhaled nitric oxide correlate with eosinophilic airway inflammation measured in induced sputum] and in bronchial lavage and biopsy. This is a correlation seen in asthma and in any condition where eosinophilic inflammation is present: for example, allergic rhinitis, eosinophilic bronchitis [10] and COPD [11]. FeNO is therefore recognized as a reliable marker of airway inflammation, and a diagnostic and management tool that can be used both in asthma diagnosis and asthma management. The clinical usefulness of exhaled nitric oxide applies to other inflammatory conditions of the lower airway.

Carbon monoxide (CO), exhaled as a function of gas exchange in the alveoli, also has diverse physiological functions. Endogenous CO can arise from the metabolism of heme by heme oxygenase [12], an enzyme whose expression and activity is enhanced in airway macrophages by either increased oxidative stress or stimulation by proinflammatory cytokines [13]. Devices measuring exhaled CO in tobacco smoke intake for smoking cessation programs have already been developed, and researchers are now creating ways to measure exhaled CO for the determination of hemolytic jaundice in neonates.

Exhaled breath condensate (non-VOC constituents)

Hundreds of compounds have been identified in condensed exhaled breath, ranging from the smallest of molecules—the hydrogen ion (H+) to large proteins such as albumin. Many of the measured compounds reflect various components of airway and lung homeostasis and disease, including airway acidification, and provide information relevant to determining presence of inflammation and oxidative injury. Although the use of EBC is a promising technique in the assessment of airways inflammation [14], it also serves as a matrix to assay systemically produced exhaled compounds, and therefore can provide information far beyond the single organ system of the lung[15-19]. Exhaled breath condensate has even been used to detect environmental exposure of workers to cobalt and tungsten [20].

Exhaled volatile organic compounds (VOC)

The term volatile organic compound refers to a group of chemicals which can vaporized easily at room temperature [21]. The most abundant VOC's are chemicals used in the production of many common household goods and supplies. For this reason VOCs are considered a source of indoor air pollution and exhaled breath analysis has been used to assess VOC exposure [22].

Although the origins of VOC analysis lie in environmental exposure testing, constituents have also been evaluated as non-invasive biomarkers of disease such as hyperlipidemia [23] and lung cancer [24]. Clinical studies are now under way to determine the effectiveness of measuring VOC's in individuals as an early marker for lung cancer.

Bringing VOC Measurement into Wider Use: The Revelar Device

One of the most interesting applications of VOC analysis can be found in the study of oxidative stress. The concept of oxidative stress is key to understanding both the pathophysiology of certain diseases and the effect of potential therapeutic interventions. Understanding oxidative stress starts with the basic tenet that each cell in our bodies is surrounded by a membrane that is made up of thousands of molecules of fatty acids, which can be attacked by excess oxygen-based free radicals, altering them leading to their dysfunction in a process called oxidative stress. This process begins with the breakdown of the fatty acids and ends ultimately with the release of a variety of aldehydes. Many different volatile aldehydes are formed during the oxidation of cell lipid membranes. These volatile aldehydes are released into blood stream and then in part exhaled out of the lungs. Measuring the exhaled aldehydes provide evidence of ongoing oxidative stress.

Many conditions and diseases are known to cause oxidative stress, including heart disease, cancer, autoimmune and neurodegenerative disease, some infectious diseases and chronic fatigue syndrome. Recently, studies using laboratory analytical methods have shown that the amounts of aldehydes in exhaled breath can be correlated with the effects of lifestyle habits such as smoking and the presence of disease states known to involve an oxidative stress pathway [25-27]. These studies have also shown that the levels of these compounds change after appropriate therapy [27]. However, these analytical methods require very expensive equipment and a high degree of technical skill, rendering them impractical for widespread use. Thus, there is a need to measure aldehydes in exhaled breath in a simple and inexpensive manner.

Current available methods that are also clinically viable include the TBARS test [28], which measures just one aldehyde [MDA]. Other methods can quantify unsaturated aldehydes, but do not measure equally relevant saturated aldehydes.

Pulse Health LLC has developed a system that measures both unsaturated and saturated aldehydes with a clinically viable device. The REVELAR System was developed as an inexpensive way to measure a panel of aldehydes, allowing for a more complete picture of the fatty acid oxidation processes occurring in the body. The system uses a proprietary reagent and a small, portable device to measure a relevant profile of aldehydes in the same type of breath sample—and do so quickly, accurately, precisely, and inexpensively.

The Pulse technology relies on a well known chemical reaction between Schiff base and aldehydes. The Schiff test, invented and named after Hugo Schiff is a chemical reaction test for the detection of aldehydes[29]. When aldehyde is added to the decolorized Schiff reagent a characteristic magenta or purple color develops. The Schiff reagent is the reaction product of Fuchsine or the closely related Pararosaniline (lacks a methyl group) and sodium bisulfite. The magnitude of color change can be correlated to the concentration of aldehyde present, forming the basis for the Pulse technology.

In addition to measuring a broad range of aldehydes, the REVELAR system provides a low cost and effective way to for practioners to instantly review and patient's oxidative stress level and lifestyle choices. The average cost of the test is $30, the colorimetric reaction is rapid, and the reader provides a score nearly immediately. Pulse is currently in the process of developing data from clinical patient visits throughout the United States and Canada.

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