CHEST

Commentary

AHEAD OF THE CURVE

Analysis of the Exhalome

A Diagnostic Tool of the Future

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Investigations on breath analysis have provided preliminary data on its potential in the noninvasive diagnosis of lung diseases. Although the conventional comparisons of exhaled breath in study populations (ie, diseased vs healthy) may help to identify patients with various lung diseases, we believe that the analysis of exhaled breath holds promise beyond this scenario. On the basis of preliminary findings, we hypothesize that breath analysis (1) could be applied not only to identify patients with lung disease but also to better phenotype healthy subjects at risk and patients with a particular disease, which is in-line with current efforts toward individualized medicine; (2) could be useful in estimating internal body time to determine the optimal time of drug administration, thereby maximizing drug activity and reducing toxicity (chronopharmacology); and (3) could be applied to monitor drugs or drug metabolites, thus, enhancing adherence to prescribed medications and enabling studies on pharmacokinetics. ***CHEST 2013; 144(3):746–749***

The fact that exhaled breath contains valuable information on the health status of a subject has been known since the time of the ancient Greeks (eg, fetor hepaticus). Breath analysis offers a unique opportunity to retrieve relevant information on ongoing internal biochemical processes noninvasively, as parts of the most volatile components of blood reach the gas phase and are subsequently exhaled (eg, detection of ethanol in breath). In addition, because the respiratory tract is in direct contact with air, it also contributes to the composition of exhaled breath. For example, simple methods of breath analysis have been used in the assessment of bronchial inflammation by measuring nitric oxide. For these reasons, and as shown by a substantial number of investigations published on this topic, analysis of breath may be a valuable tool in diagnosing not only lung diseases but also other conditions that disturb metabolism, such as diabetes and renal or liver failure. However, despite this appealing approach, breath analysis is still in its infancy.[1] Compared with the clinical analysis of other body fluids and tissue specimens, exhaled breath is still a far way from reaching the maturity needed to widely support clinicians, but major steps forward have been made during the past decade in an attempt to improve this situation. For example, major technological developments have enabled the detection of a wide range of analytes in breath, and importantly, this can be achieved in real time.[2,3] Relevant metabolites such as isoprene,[2,3] methanol,[2,3] acetone,[2,3] urea,[4] free fatty acids,[5] and a number of yet unidentified compounds have been reported to be detectable in human breath.[6] Figure 1 schematically shows a subject breathing into a mass spectrometer; in only 3 min, three replicate measurements with excellent repeatability provide breath mass spectrometric fingerprints. In addition, modern mass spectrometry not only is suitable for breathprinting but also allows a structural elucidation of the compounds detected in breath. For example, the trace shown in Figure 1 illustrates the breath-to-breath (three replicated measurements) detection of indole (tryptophan metabolism, C_8H_7N, molecular structure displayed) in human exhaled breath.

Although the number of detectable compounds in real time typically is smaller than the one based on off-line analysis of blood, urine, or tissue specimens,

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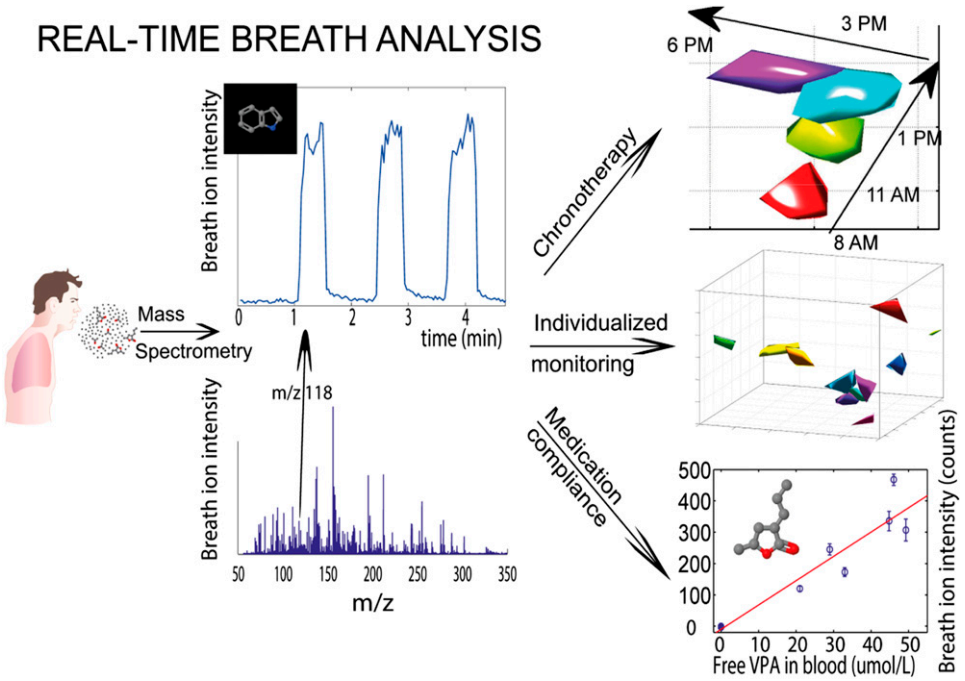

FIGURE 1. Three potential clinical scenarios where real-time, in vivo analysis of exhaled breath may become an important tool. A subject is depicted exhaling into a mass spectrometer. As a result, mass spectrometric breathprints similar to the one shown are collected in real time. The plot above the mass spectrum illustrates the online detection of indole (tryptophan metabolism; structure shown in the inset) in breath (m/z, 118). Three replicate exhalation measurements are typically performed within 3 min. The mass spectrometric breath signatures are subsequently analyzed statistically to address three different themes. The first graph illustrates how the human breath composition evolves during the course of a day; this may have the potential to estimate the internal body time. The second graph suggests the existence of individual exhaled phenotypes. Such individualized breathprints may support current techniques toward personalized health care. The third graph presents the findings of a pilot study showing the correlation between an exhaled metabolite of VPA (structure shown in the inset) and blood concentration of VPA. It suggests that a direct estimation of adherence to medication may be feasible through analysis of exhaled breath. m/z = mass-to-charge ratio; VPA = valproic acid.

the breathprints (exhalome) obtained by the latest techniques seem to be informative enough to explore new fields, other than the diagnosis of diseases, rapidly, in vivo, and noninvasively. Here, we briefly describe three major areas where breath analysis may contribute significantly in the future.

Open Issues in Medicine: Internal Body Time, Individualized Treatment, and Adherence to Medication

Estimation of Internal Body Time

Biologic clocks play a major role in living systems, with serious implications regarding drug activity and toxicity depending on the delivery time.[7] For this reason, chronobiology aims to estimate internal body time based on the characterization of the fluctuating metabolome in a circadian fashion.[8] We hypothesize that just as part of the plasma metabolome oscillates around the clock, so may fluctuations in human breath composition. If these temporal events could be captured, a model to predict the individual internal body time that is similar to plasma-based methods but noninvasive could be created. Before testing this hypothesis through a rigorous sampling schedule around the clock during several days, we have shown that a core of compounds in human breath exhibits a routine daily pattern in measurements taken during 9 nonconsecutive days in 12 subjects.[9] Figure 1 (first graph) shows a model created with >200 human breath mass spectra during 9 days. The results strongly suggest that internal body time may be tracked through the analysis of exhaled breath.

Individual Phenotypes

The metabolome ultimately released into body fluids reflects a combination of internal (eg, genome) and external (eg, environmental) factors.[10] To address the paradigm of individualized treatment, whereby medical therapy is precisely tailored to the specific needs of subpopulations or even individuals, the impact of these internal and external factors need to be understood. Therefore, tremendous efforts are being devoted to identify individual metabolic phenotypes. For example, it has been shown that individual urine metabolic

phenotypes seem to be stable for several years.[11,12] Similarly, we hypothesize that if there are highly individual metabolic traits in urine, characteristic individual exhalome phenotypes may also exist. The model shown in Figure 1 (second graph) shows the three-dimensional projection of the breath mass spectra of 11 healthy subjects during 9 days.[13] Clustering according to donor (color-coded) is obvious, suggesting that a stable individual signature of breath exists.

One possible scenario is that such individualized breath mapping is performed in healthy subjects at risk for a specific disease. These subjects may conceivably undergo breath analysis in addition to traditional tests during routine medical examinations. If during one of these control measurements, the subject provides a breath mass spectrum that falls a far way from the natural individual region in the model, such unusual deviation (well beyond that expected from normal daily variance) may be considered an early warning. Additionally, one could envisage the construction of an individualized breathprint to monitor disease progression and the (side) effects of therapy.

Adherence to Medication

Adherence to medication is a major concern for clinicians and has an enormous economic and social impact.[14] Methods to evaluate adherence to medication usually include the direct analysis of the drug or one of its metabolites in blood or urine.[15] However, such blood- or urine-based methods usually are expensive and time consuming. Despite these drawbacks, they are the most reliable procedures available today to estimate adherence to medication.[15] We hypothesize that breath analysis may contribute to tackling this issue in a rapid, noninvasive, and possibly inexpensive way. For example, the third graph in Figure 1 shows the signal intensity of a valproic acid metabolite (molecular structure shown in the inset) present in breath vs free valproic acid concentration in blood in six patients with epilepsy taking valproic acid medication compared with three healthy control subjects.[16] The correlation found between valproic acid in blood and its metabolite in breath suggests that in vivo monitoring of exhaled metabolites to assess adherence to medication and monitor pharmacokinetics may be a feasible approach.

Testing the Hypothesis

Major technological advancements during the past years in the field of metabolomic profiling of body fluids have unlocked new potential applications in clinical medicine.[17] We hypothesize that even though exhaled breath contains less metabolic information than liquid-phase body fluids simply because metabolites with negligible vapor pressure cannot reach the gas phase, breath still contains enough biochemical information that can be assessed in great detail in vivo with state-of-the-art instrumentation. This may help to address some of the major open questions in modern medicine.

In particular, we hypothesize that the in vivo analysis of exhaled breath may support clinicians in (1) determining the preferable or optimum time to administer a drug by predicting an individual's internal body time, (2) sounding a warning when the individual exhalome deviates substantially from normal values as a result of causes other than routine external factors, and (3) estimating medication adherence and monitoring pharmacokinetics. To test each of these three hypotheses will require long-term studies that involve large, heterogeneous, and well-characterized cohorts of patients and healthy control subjects. To conduct such studies beyond mere pilot investigations, the mass spectrometric technology, which usually is only available in basic research institutions, will have to be transferred to the clinic. In addition, the successful transfer of this method into the clinical setting will require the joint expertise of multidisciplinary groups, including basic research scientists, (chrono) biologists, statisticians, and clinicians.

Conclusion

We are still far from fully exploiting the entire potential of exhaled breath analysis. However, if the scenario that we hypothesize is true, it will have a significant impact on current clinical medicine not only by supporting the process of diagnosis but also by adding to (1) chronotherapy, (2) individualized health care, and (3) adherence to medication and monitoring of drug pharmacokinetics.

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