The Breathprints in Patients With Liver Disease Identify Novel Breath Biomarkers in Alcoholic Hepatitis

Ibrahim A. Hanouneh,[*] Nizar N. Zein,[*] Frank Cikach,[‡] Luma Dababneh,[‡] David Grove,[‡] Naim Alkhouri,[*] Rocio Lopez,[§] and Raed A. Dweik[‡]

[*]Department of Gastroenterology and Hepatology, [‡]Department of Pulmonary, Allergy, and Critical Care Medicine/Respiratory Institute, and [§]Department of Quantitative Health Science, Cleveland Clinic, Cleveland, Ohio

BACKGROUND & AIMS: **Selected-ion flow-tube mass spectrometry can precisely identify trace gases in the human breath, in the parts-per-billion range. We investigated whether concentrations of volatile compounds in breath samples correlate with the diagnosis of alcoholic hepatitis (AH) and the severity of liver disease in patients with AH.**

METHODS: **We recruited patients with liver disease from a single tertiary care center. The study population was divided between those with AH with cirrhosis (n = 40) and those with cirrhosis with acute decompensation from etiologies other than alcohol (n = 40); individuals without liver disease served as control subjects (n = 43). We used selected-ion flow-tube mass spectrometry to identify and measure 14 volatile compounds in breath samples from fasted subjects. We used various statistical analyses to compare clinical characteristics and breath levels of compounds among groups and to test the correlation between levels of compounds and severity of liver disease. Logistic regression analysis was performed to build a predictive model for AH.**

RESULTS: **We identified 6 compounds (2-propanol, acetaldehyde, acetone, ethanol, pentane, and trimethylamine [TMA]) whose levels were increased in patients with liver disease compared with control subjects. Mean concentrations of TMA and pentane (TAP) were particularly high in breath samples from patients with AH, compared with those with acute decompensation or control subjects (for both, $P < .001$). Using receiver operating characteristic curve analysis, we developed a model for the diagnosis of AH based on breath levels of TAP. TAP scores of 36 or higher identified the patients with AH (area under the receiver operating characteristic curves = 0.92) with 90% sensitivity and 80% specificity. The levels of exhaled TMA had a low level of correlation with the severity of AH based on model for end-stage liver disease score ($r = 0.38$; 95% confidence interval, 0.07–0.69; $P = .018$).**

CONCLUSIONS: **Based on levels of volatile compounds in breath samples, we can identify patients with AH vs patients with acute decompensation or individuals without liver disease. Levels of exhaled TMA moderately correlate with the severity of AH. These findings might be used in diagnosis of AH or in determining patient prognosis.**

Keywords: Marker Panel; Liver Damage; Microbiota; Alcohol Consumption.

See editorial on page 524.

Liver biopsy remains the gold standard for the assessment of hepatic fibrosis and cirrhosis and is helpful in determining the prognosis and management of chronic liver disease. However, liver biopsy is an invasive procedure, and it carries a risk of complications. Indeed, 1%–5% of patients require hospitalization after the procedure.[1] Furthermore, sampling error and interobserver variability add to the limitations of liver biopsy.[2] Therefore, there is an increasing demand for alternative noninvasive methods to assess the severity of liver disease.

The clinical use of breath as a medical tool in the diagnosis of chronic liver disease has been reported many years ago in the description of fetor hepaticus "a

Abbreviations used in this paper: **AD, acute decompensation; AH, alcoholic hepatitis; AUC, area under the ROC curves; CI, confidence interval; CPS, Child-Pugh score; FMO, flavin monooxygenase; MDF, Maddrey's discriminant function; MELD, model of end-stage liver disease; ROC, receiver operating characteristic; SIFT-MS, selected-ion flow-tube mass spectrometry; SIM, selected ion monitoring; TAP, TMA and pentane; TMA, trimethylamine; TMAO, TMA N-oxide.**

1542-3565/$36.00
http://dx.doi.org/10.1016/j.cgh.2013.08.048

distinctive musty, sweet breath odor in individuals with severe liver disease."[3] With recent advances in technology, it is possible to identify thousands of substances in the breath, such as volatile compounds and elemental gases.[3] Using selected-ion flow-tube mass spectrometry (SIFT-MS), precise identification of trace gases in the human breath in the parts-per-billion ranges can be achieved.[4,5]

A recent study has identified a novel pathway linking dietary lipid intake, intestinal microflora, and atherosclerosis.[6] Researchers showed that intestinal microflora plays an important role in the formation of trimethylamine (TMA) from dietary phosphatidylcholine and dietary free choline (Figure 1). The hepatic flavin monooxygenase (FMO) family of enzymes, specifically FMO3, converts TMA, a volatile organic compound that smells like rotting fish, into TMA N-oxide (TMAO), an odorless stable oxidative product that contributes to atherosclerosis in humans.[6]

Subjects with chronic liver disease have impaired capacity to convert TMA into TMAO.[7] Furthermore, small intestinal motility dysfunction and small intestinal bacterial overgrowth, commonly seen in patients with liver cirrhosis, creates a favorable environment for translocation of the enteric bacteria to the systemic circulation.[8,9] This, in addition to alcohol consumption, induces bacterial overgrowth and increases gut permeability and the translocation of bacteria-derived lipopolysaccharides from the gut to the liver in patients with chronic liver disease.[8,9] These may ultimately contribute to the increased levels of TMA in patients with chronic liver disease, in general, and alcoholic liver disease, in particular.

We therefore sought to determine whether the concentration of volatile compounds in the breath correlates with the diagnosis and the severity of liver disease. We aimed to assess the accuracy of measuring TMA in the breath using SIFT-MS in predicting the diagnosis and the severity of alcoholic hepatitis (AH).

Patients and Methods

After receiving approval from the Institutional Review Board at Cleveland Clinic, Cleveland, Ohio, we prospectively recruited patients with liver disease who were admitted to the liver inpatient service at Cleveland Clinic between February 2011 and February 2013. The study population was divided into 2 groups: group 1, patients with liver cirrhosis and AH; and group 2, patients with liver cirrhosis and acute decompensation (AD) from etiologies other than alcohol. A healthy group without liver disease was identified to serve as a control group.

The diagnosis of AH was made based on the presence of the following laboratory criteria[10] in a patient with a history of heavy alcohol use after excluding other causes of liver disease: (1) aspartate aminotransferase level that is elevated, but <300 IU/mL; (2) ratio of aspartate aminotransferase level to alanine aminotransferase level that is >2; (3) total serum bilirubin level of >5 mg/dL; (4) an elevated international normalized ratio; and (5) neutrophilia. Liver biopsy was considered when the diagnosis of AH was uncertain.[10] Significant alcohol intake was defined as a consumption of >2 drinks

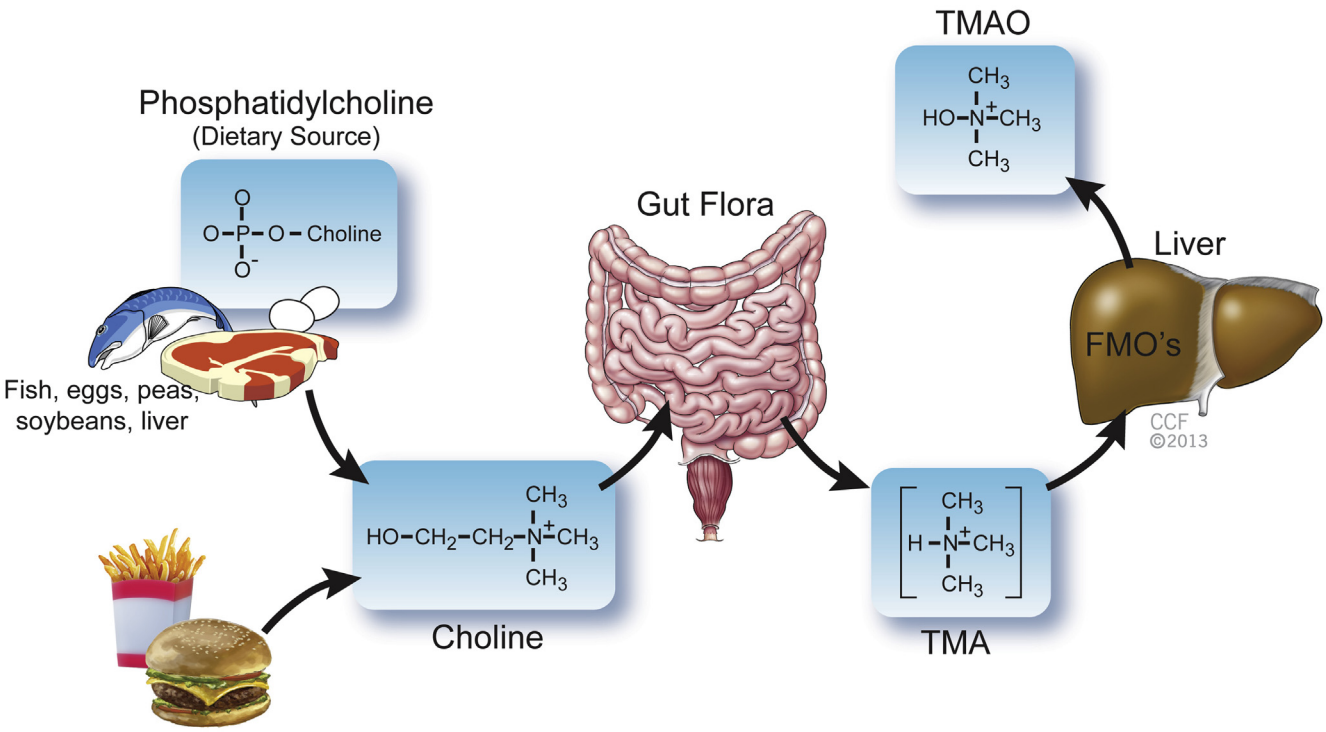

Figure 1. Metabolism of dietary phosphatidylcholine. Reprinted with permission, Cleveland Clinic Center for Medical Art & Photography © 2013. All Rights Reserved.