Published in final edited form as:

Clin Gastroenterol Hepatol. 2014 March ; 12(3): 516–523. doi:10.1016/j.cgh.2013.08.048.

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

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Conflict of Interest:

- Ibrahim Hanoun: None to declare.
- David Grove: None to declare.
- Luma Dababneh: None to declare.
- Naim Alkhouri: None to declare.
- Rocio Lopez: None to declare.
- Frank Cikach: None to declare.
- Nizar Zein: None to declare.
- Raed Dweik: None to declare.

Authors Financial Disclosure:

- Ibrahim Hanoun: No financial relationship with a commercial interest.
- David Grove: No financial relationship with a commercial interest.
- Luma Dababneh: No financial relationship with a commercial interest.
- Naim Alkhouri: No financial relationship with a commercial interest.
- Rocio Lopez: No financial relationship with a commercial interest.
- Frank Cikach: No financial relationship with a commercial interest.
- Nizar Zein: No financial relationship with a commercial interest.

Role of each Author in the manuscript:

- Ibrahim Hanoun: study concept and design, recruiting patients, acquisition of data, drafting of the manuscript.
- Naim Alkhouri: study concept and design, drafting of the manuscript.
- Frank Cikach: obtaining breath samples, and running the breath analysis.
- Rocio Lopez: statistical analysis.
- Nizar Zein: critical revision of the manuscript for important intellectual content, study supervision.
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Abstract

Background & Aims—Selected-ion flow-tube mass spectrometry (SIFT-MS) 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 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 controls (n=43). We used SIFT-MS 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 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 controls. Mean concentrations of TMA, acetone, and pentane were particularly high in breath samples from patients with AH, compared to those with acute decompensation or controls (for both, $P<.001$). Using receiver operating characteristic curve analysis, we developed a model for the diagnosis of AH based on breath levels of TMA, acetone, and pentane (TAP). TAP scores of 36 or higher identified the patients with AH (AUC=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; $P=.018$).

Conclusion—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

Introduction

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 to 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 distinctive musty, sweet breath odor in individuals with severe liver disease”. 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 (ppb) 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 which smells like rotting fish, into trimethylamine N-oxide (TMAO), an odorless stable oxidative product which 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, that 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 with the severity of liver disease. We aimed, in particular, 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 our institution, 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 two groups: group-1: patients with liver cirrhosis and AH; and group-2: patients with liver cirrhosis and acute de-compensation (AD) from etiologies other than alcohol. A healthy group without liver disease was identified to serve as 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 per milliliter; 2) ratio of aspartate aminotransferase level to alanine aminotransferase level that is more than 2; 3) total serum bilirubin level of more than 5 mg per deciliter; 4) an elevated INR, and 5) neutrophilia. Liver biopsy was considered when the diagnosis of AH was uncertain [10]. “Significant alcohol intake” was defined as a consumption of more than two drinks daily or more than six drinks daily on weekends for the past five years. We used the definition of American Association for the study of liver disease guidelines of what constitutes a standard drink – 12 gram of alcohol with range 9.3–13.2 gram.

The diagnosis of liver cirrhosis was identified based on the histological features of cirrhosis on liver biopsy and/or a composite of clinical signs and findings of cirrhosis provided by laboratory tests, endoscopy and radiologic imaging. AD was defined by the acute development of one major complication of liver disease including acute kidney injury, ascites, encephalopathy, or gastrointestinal hemorrhage secondary to gastrointestinal varices or portal hypertensive gastropathy/enteropathy. Hepatic encephalopathy was assessed by a single individual using Conn score and asterix grade. Acute kidney injury was defined as

an abrupt (arbitrarily set at 48 hours) reduction in kidney function manifested by an absolute increase in serum creatinine of 0.3 mg/dL or more, equivalent to a percentage increase in serum creatinine of 50% or more (1.5-fold from baseline).

Among patients in study group-2 with AD of liver cirrhosis, only those who were alcohol sober for at least six months prior to admission were included, whereas all patients with AH were (by definition) actively abusing alcohol prior to admission. Patients with severe hepatic encephalopathy who were unable to provide consent or breath sample at time of admission were excluded from the study. We also excluded all individuals with ongoing tobacco use. Patients with liver cancer or other malignancies were excluded as well as those with prior history of transplantation.

SIFT-MS breath analysis was performed on all subjects to measure volatile organic compounds (VOCs) in the exhaled breath. Breath samples were collected in the fasting state upon admission to the hospital in patients with liver disease before the clinical/histological diagnosis was made. Blood samples were also obtained at the time of the breath test and used to measure complete blood count, basic metabolic panel, liver function tests and prothrombin time and INR. The Model of End-Stage Liver Disease (MELD) score, Child's Pugh score (CPS) and Maddrey's Discriminant Function (MDF) were calculated.

Exhaled Breath Collection

All subjects completed a mouth rinse with water prior to the collection of the breath sample in order to reduce the contamination from VOCs produced in the mouth. Subjects were prompted to exhale normally to release the residual air from the lungs and then inhale to total lung capacity through a disposable mouth filter. The inhaled ambient air was also filtered through an attached N7500-2 acid gas cartridge. The filters were used to prevent viral and bacterial exposure to the subjects and to eliminate exogenous VOCs from the inhaled air. The subjects then proceeded to exhale at a rate of 50 ml/second through the mouth filter until the lungs were emptied. The exhaled breath samples were collected into an attached Mylar® bag, capped, and analyzed within four hours using Selected-Ion Flow-Tube Mass Spectrometry (SIFT-MS). Mylar® bags were cleaned by flushing with nitrogen between subjects.

Selected-Ion Flow-Tube Mass Spectrometry (SIFT-MS)

The exhaled breath samples underwent gas analysis using SIFT-MS on a VOICE200® SIFT-MS instrument (Syft Technologies Ltd, Christchurch, New Zealand). The SIFT-MS technology and instrument used in this study have previously been described and validated elsewhere by our group and others [11–12].

Mass scans of the product ions generated in the chemical ionization mass spectrum from each reagent ion (H_3O^+ , NO^+ , and O_2^+) were obtained in the mass scanning (MS) mode. MS mode, between 14–200 amu, was used to identify significant peaks at product ion masses representing unknown breath volatiles relating to liver disease. More accurate concentration data was obtained by selected ion monitoring (SIM) of product ions of fourteen pre-selected compounds: 2-propanol, acetaldehyde, acetone, acrylonitrile, ammonia, benzene, carbon disulfide, dimethyl sulfide, ethanol, hydrogen sulfide, isoprene, pentane, triethylamine, and TMA. These compounds have been previously identified as common constituents of exhaled breath in patients with and without liver disease [11].

The MS data was normalized to account for variability in the precursor ion intensity by dividing each mass signal by the sum of the corresponding precursor ion signals. Normalization was not required for the SIM data because absolute concentrations of SIM

analytes were calculated from the precursor ion to product ion count ratios by the built-in LabSyft software and libraries.

Statistical analysis

Data are presented as mean $\pm$ standard deviation, median [25th, 75th percentiles] or N (%). Univariable analysis was performed to compare the clinical characteristics and the breath compound levels between the 3 groups. Analysis of variance (ANOVA) or the non-parametric Kruskal-Wallis test was used to assess differences in continuous variables and Pearson's chi-square tests were used for categorical factors. In addition, analysis of covariance (ANCOVA) was used to assess differences in compound levels while adjusting for age and gender; the logarithm of each compound was modeled as the outcome variables with group, age and gender as the independent variables. This was also done after excluding subjects with AH who had not had a liver biopsy done. Also, Spearman's correlation coefficients were used to assess correlations between breath compounds and severity of liver disease. Logistic regression analysis was performed to build a predictive model for AH; AH vs. AD was modeled as the outcome and the difference compounds were considered for inclusion. Receiver Operating Characteristics (ROC) analysis was performed to assess the role of breath compounds in distinguishing between AH and AD; the area under the ROC curves (AUC) and corresponding 95% confidence intervals are presented and DeLong's method was used to compare the AUCs. The logistic regression model with the highest AUC is presented. Bootstrap validation performed on 500 bootstrap samples was performed to validate the model; the optimism corrected AUC is presented. To account for multiple comparisons a significance criterion of 0.004 (0.05/14) was used for the univariable analysis of the breath compounds, otherwise a $P < 0.05$ was considered statistically significant. SAS (version 9.2, The SAS Institute, Cary, NC) and R (version 2.15.2, The R Foundation for Statistical Computing) were used to perform all analyses.

Results

Patients Characteristics

A total of eighty patients were included in the study, of whom forty were admitted with AH in the background of cirrhosis, and forty subjects with AD of liver cirrhosis. Forty-three healthy subjects without liver disease were identified and served as a control group.

Patients with liver disease were older than healthy control (51.4 ± 11.4 vs. 37.9 ± 13.6 years, $p < 0.001$), and predominantly males [43(53.8%) vs. 14(32.6%), $p = 0.025$]. The demographic and clinical characteristics of the study population are shown in Table-1.

Among patients with AD, 20 had cirrhosis secondary to remote history of alcohol abuse. The other 20 AD patients had liver cirrhosis secondary to the following etiologies [non-alcoholic steatohepatitis (n=8), chronic hepatitis C (n=7), chronic hepatitis B (n=1), alpha-1 antitrypsin deficiency (n=2), primary sclerosing cholangitis (n=1) and cardiac cirrhosis (n=1)].

Among patients with AH, liver biopsy was obtained to confirm the diagnosis in 21(53%) patients. All patients with AH had liver cirrhosis based on the histological features and/or radiological evidence of cirrhosis in the context of manifestations of portal hypertension (ascites, encephalopathy, variceal bleed or thrombocytopenia). Seventy-two percent of patients with AH had severe disease as defined by MDF score greater than 32, of whom 23% were treated with corticosteroids, 18% with pentoxifylline, and 31% with combination of corticosteroids and pentoxifylline.

At the time of enrollment, the baseline MELD score in the AH patients was 23.5 ± 8.8 , compared to 19.3 ± 8.0 in AD patients ($p=0.03$). This was driven by remarkably higher bilirubin values in patients with AH compared to AD [$14.2(8.4, 23.3)$ vs. $4.0(2.0, 8.7)$ mg/dL, $p<0.001$]. The CPS of patients with AH and AD were closely similar (10.1 ± 1.9 vs. 10.2 ± 1.9 , $p=0.69$). Hepatic encephalopathy and ascites were equally present between AH and AD patients. Additionally, the uses of lactulose or rifaximin at the time of enrollment were not different between the two groups (Table-1).

Breathprints in Patients with Liver Disease

Of the fourteen pre-selected SIM compounds, we identified six exhaled breath compounds that were elevated in patients with liver disease compared to healthy control (Table-2A). Those compounds include: 2-propanol, acetaldehyde, acetone, ethanol, pentane, and TMA. There were no differences in the breath levels of benzene, acrylonitrile, carbon disulfide, dimethyl sulfide, isoprene, ammonia, hydrogen sulfide or triethylamine in patients with liver disease compared to healthy control (Table-2A).

Of the six breath compounds that were higher in patients with liver disease compared to healthy control, there were four breath compounds that stood out in patients with AH. The breath concentrations of acetaldehyde, TMA, acetone and pentane were significantly higher in patients with AH compared to those with AD, while the concentrations of other volatile compounds (2-propanol, acrylonitrile, carbon disulfide, dimethyl sulfide, ethanol, isoprene, ammonia, hydrogen sulfide or triethylamine) in the exhaled breath were not different between patients with AH and those with AD (Table-2B).

Breath-Biomarkers in AH

Development of TAP Score – A Potential Tool for Diagnosis of AH—After adjusting for age and gender, the concentrations of TMA, acetone and pentane in the breath were significantly higher in patients with liver cirrhosis and AH compared to those with liver cirrhosis and AD, and compared to healthy volunteers (Table-2B). The three breath compounds remained associated with AH after adjusting for infection. Furthermore, the associations between TMA and pentane in the exhaled breath and AH remained significant after accounting for MELD score (TMA: $p<0.001$, pentane: $p=0.004$); but the concentration of acetone in the breath did not reach a statistically significant value ($p=0.07$). The significance of the breath's acetone, pentane and TMA in patients with AH remained the same after excluding patients with clinical diagnosis but no histological confirmation of AH (all $p<0.001$).

Figure-2 presents the results of the ROC analysis. A cutoff value of breath TMA of 14 ppb provided 90% sensitivity and 72% specificity for the diagnosis of AH [AUC 95%CI: $0.887(0.815, 0.959)$]. Breath's acetone and pentane were less sensitive but more specific for the diagnosis of AH. A breath acetone value of 489 ppb was 60% sensitive and 92% specific [AUC 95%CI: $0.778(0.673, 0.883)$]; and a breath pentane value of 30 ppb was 57% sensitive and 95% specific in the diagnosis of AH. A combination of pentane and TMA levels in the breath was found to provide excellent prediction accuracy for the diagnosis of AH (AUC=0.92); this was true after performing bootstrap validation (Optimism corrected AUC=0.90).

The final logistic regression (lr) function combining two breath variables; TMA, and Pentane for the diagnosis of AH was [$\text{lr} = -3.71 + (0.34 * \text{TMA}) - (0.087 * \text{Pentane})$]. The logistic function (lr) value was then converted into a probability distribution with a value between 0 to 100 and called "TAP score" by the following formula: $\text{TAP score} = 100 \times [\exp(\text{lr}) / (1 + \exp(\text{lr}))]$. A TAP score of 36 provided 90% sensitivity and 80% specificity for the

diagnosis of AH; and a TAP score of 67 was 70% sensitive and 94% specific for the diagnosis of AH.

Breathprint and Severity of AH

There was a moderate correlation between the concentrations of TMA in exhaled breath and severity of liver disease in patients with AH as presented by MELD score [ρ (95%CI); 0.38(0.07, 0.69), $p=0.018$]. This is largely driven by the association between breath TMA and serum creatinine [ρ (95%CI); 0.36(0.05, 0.67), $p=0.026$]. There was no correlation between concentrations of breath TMA and either serum bilirubin [ρ (95%CI); 0.28(−0.03, 0.60), $p=0.079$] or INR [ρ (95%CI); 0.23(−0.09, 0.56), $p=0.15$]. Therefore, the association between TMA levels in breath and MDF score [ρ (95%CI); 0.30(−0.02, 0.62), $p=0.062$], and CPS [ρ (95%CI); 0.17(−0.16, 0.50), $p=0.31$] did not reach statistically significant values (Table-3).

Similarly, the associations between the breath's pentane and acetone and the MELD score in AH subjects were not statistically significant [pentane: 0.30(−0.01, 0.62), $p=0.06$ and acetone: 0.29(−0.03, 0.61), $p=0.07$] (Table-3).

Discussion

To date, the diagnosis of AH is made clinically based on a typical presentation, with severe liver dysfunction in the context of excessive alcohol consumption, and the exclusion of other causes of acute and chronic liver disease. However, it has been shown that the physician's clinical impression may correlate only moderately well with the diagnosis of AH. Studies that have included a liver biopsy in all patients with clinically suspected AH have shown histological confirmation in only 70%–80% of patients [13]. Therefore, in the absence of non-invasive alternatives, liver biopsy remains today the gold standard for the diagnosis of AH.

Our study showed that patients with AH have specific pattern of breathprint that is characterized by high levels of TMA, acetone and pentane in the exhaled breath compared to patients with AD of liver disease from etiologies other than alcohol. Using the combination of the breath levels of TMA, Acetone and Pentane, this study presents the TAP model, as a potential new tool complementary or alternative to the standard clinical and histological methods in assessing the diagnosis of AH. The TAP model provided excellent prediction accuracy for the diagnosis of AH with 97% sensitivity and 72% specificity for a TAP score of 28; and 80% sensitivity and 86% specificity for a TAP score of 51.

In 1951, Soderstrom [14] noted that patients suffering from severe liver disease when treated with choline spread an unpleasant odour and that their urine had a very disagreeable smell. A sample of such urine was sent for chemical analysis and TMA was actually isolated. In agreement with this finding, Mitchell et al [15] examined the urine of 63 patients with various liver diseases. In total, 50% of the patients had elevated levels of urinary TMA. Seventeen patients excreted large amounts of free TMA in the urine (>10 microg/ml), above the threshold usually associated with the appearance of a 'fish-like' body odor and tainted breath. It remained unknown, however, that why only a subgroup of patients with liver disease excretes large amount of TMA. According to our data, it is a subgroup of cirrhotic patients with AH.

The observation of elevated levels of TMA in patients with AH may suggest that in AH, the transformation of TMA to TMAO might be impaired as a consequence of damaged liver function. This theory is based on the recognition of the etiology of an uncommon genetic disorder called trimethylaminuria - also known as fish malodour syndrome [16]. Subjects

with this metabolic condition have defective FMO3 enzyme; and as such, impaired capacity to convert TMA into TMAO. Genome-wide association studies have been recently successful in finding the gene of FMO3 enzyme [17], which is located in chromosome region 1q23–25. In agreement with this hypothesis, a study by Wranne et al has indicated that the extent of liver damage is accompanied by a reduced capacity of the liver to transform TMA to TMAO [14]. In this study, Wranne et al had examined the urinary excretion of TMA and TMAO following the administration of TMA to healthy individual and to patients with liver disease. The ratio of TMAO/TMA was markedly reduced in patients with severe liver dysfunction, a finding which suggests that the physiological oxidation of TMA is impaired in patients with liver disease. Our data showed that the levels of exhaled breath TMA moderately correlate with severity of liver disease in AH as presented by MELD score. It is yet to be determined whether TMA has any detrimental effect on the liver cells or just represents a marker of severe liver dysfunction in patients with AH.

Another theory is based on the fact that TMA is derived from degradation of dietary phosphatidylcholine and dietary free choline by the intestinal microflora. Alcohol consumption in patients with alcoholic liver disease induces bacterial overgrowth and increases gut permeability and translocation of bacteria-derived lipopolysaccharides from gut to liver [8–9]. These may ultimately contribute to increased levels of TMA in patients with AH.

Pentane, another breath biomarker of AH, has been shown to be produced by lipid peroxidation based on studies with mouse liver that is exposed to carbon tetrachloride, a known hepatotoxin [18]. Alcohol ingestion increases the excretion of markers of oxidative stress; the highest levels are observed in persons with AH [19]. Studies in rats and mice suggest that free radicals are produced in liver in response to exposure to alcohol [20]. These free radicals mediate alcohol-induced liver injury, at least in part, through lipid peroxidation – a reaction that leads to production of pentane in AH.

Acetone is produced by the hepatocytes from excess acetyl-CoA. Acetone and other ketone bodies diffuse from hepatocytes into blood stream and are oxidized via Krebs cycle in peripheral tissue. Alcohol is metabolized in hepatocytes through oxidation to acetaldehyde, and subsequently from acetaldehyde to acetate [10]. Therefore, it's possible that in patients with AH the rate of production of ketone bodies exceeds the rate of utilization by peripheral tissues and the subject becomes ketonemic (elevated acetone).

Our results are limited by relatively small number of patients. Future larger studies are needed to confirm the diagnostic value of breathprint in AH. Secondly, AH was diagnosed based on the clinical ground with no histological confirmation in almost 50% of patients. Nevertheless, the significance of volatile breath compounds in AH remained the same after excluding AH patients who did not have liver biopsy. Thirdly, since all patients with AH had liver cirrhosis, our results could not be validated to patients with AH in the absence of cirrhosis. Lastly, by excluding smokers, our results cannot be generalized to patients with AH who actively smoke tobacco.

In summary, the breathprint may provide a non-invasive method for diagnosis of AH and for assessment of severity and prognosis of AH. Larger studies are needed to validate these findings, and to examine the prognostic values of breathprint in patients with AH.

Acknowledgments

Grant Support: This work was supported by BRCP 08-049 Tech 09-003 Third Frontier Program grant from the Ohio Department of Development (ODOD). Dr Dweik is also supported by the following grants: HL107147, HL081064, HL103453, HL109250, and RR026231 from the National Institutes of Health (NIH).

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Figure 1.
Metabolism of dietary phosphatidylcholine

Figure 2.
ROC Analysis of the Breathprint for the Diagnosis of Alcoholic Hepatitis. TMA= trimethylamine

Table-1

Demographic and clinical characteristics

Factor	Cirrhosis with Acute De- compensation (N=40)	Cirrhosis with Alcoholic Hepatitis (N=40)	p-value
Male	26(65.0)	17(42.5)	0.044
Age (years)	55.8±10.3	47.0±11.0	<0.001
Caucasian	36(90.0)	37(92.5)	0.99
ALK (U/L)	111.0[90.0,165.0]	156.0[97.0,212.0]	0.075
AST (U/L)	49.5[37.5,73.5]	100.0[77.0,142.0]	<0.001
ALT (U/L)	27.0[21.0,36.0]	47.0[30.0,65.0]	<0.001
Bilirubin (mg/dL)	4.0[2.0,8.7]	14.2[8.4,23.3]	<0.001
Albumin (g/dL)	2.9±0.65	3.0±0.61	0.43
INR	1.4[1.2,1.8]	1.6[1.3,1.9]	0.19
Creatinine (mg/dL)	0.93[0.77,1.6]	0.75[0.54,1.6]	0.029
MELD Score	19.3±8.0	23.5±8.8	0.03
Ascites	27(67.5)	22(56.4)	0.31
Hepatic Encephalopathy	26(65.0)	27(67.5)	0.88
Child's Pugh Score	10.2±1.9	10.1±1.9	0.69
Child's Pugh Class			
. B	14(35.0)	16(40.0)	0.52
. C	26(65.0)	22(57.9)	
Lactulose at time of breath collection	7 (17.5)	6 (15.0)	0.81
Rifaximin at time of breath collection	5 (12.5)	4 (10.0)	0.36

Values presented as Mean ± SD with ANOVA; Median [P25, P75] with Kruskal-Wallis test, or N (%) with Pearson's chi-square test.

ALK= Alkaline phosphatase, AST=Asparate amino-transferase, ALT=alanine amino-transferase, PT=Prothrombin time, INR=international normalized ratio, MELD=Model for end-stage liver disease.

Table-2

A: Breath compounds in healthy control and patients with liver disease – Age and Gender adjusted values			
Dependent	Healthy Control (N=43)	Liver Patients* (N=80)	p-value
2-propanol	34.0 (27.1, 42.7)	70.5 (60.6, 82.0)	<0.001
acetaldehyde	22.8 (18.1, 28.7)	48.8 (41.9, 56.8)	<0.001
acetone	77.5 (51.4, 116.8)	389.5 (297.1, 510.6)	<0.001
acrylonitrile	0.63 (0.55, 0.72)	0.74 (0.68, 0.81)	0.053
benzene	3.2 (2.3, 4.4)	2.9 (2.3, 3.6)	0.63
carbon disulfide	1.9 (1.5, 2.4)	2.3 (2.0, 2.7)	0.18
dimethyl sulfide	1.3 (1.01, 1.7)	1.6 (1.4, 1.9)	0.22
ethanol	44.8 (33.5, 59.8)	129.0 (106.5, 156.2)	<0.001
isoprene	12.8 (9.7, 16.9)	17.2 (14.3, 20.7)	0.1
pentane	9.6 (7.4, 12.3)	27.1 (23.0, 32.1)	<0.001
ammonia	62.5 (54.9, 71.1)	68.0 (62.4, 74.1)	0.31
hydrogen sulfide	0.37 (0.26, 0.52)	0.43 (0.34, 0.53)	0.49
triethyl amine	0.70 (0.53, 0.92)	0.89 (0.74, 1.07)	0.18
trimethyl amine (TMA)	4.1 (3.0, 5.6)	20.7 (16.7, 25.8)	<0.001

B: Breath Compounds in the patients with alcoholic hepatitis versus those with acute de-compensation – Age and Gender adjusted values			
Dependent	Cirrhosis with Acute De-compensation (N=40)	Cirrhosis with Alcoholic Hepatitis (N=40)	p-value
2-propanol	72.5 (59.8, 87.8)	72.0 (59.6, 87.0)	0.96
acetaldehyde	41.2 (33.3, 51.0)	57.0 (46.2, 70.4)	0.043
acetone	188.5 (133.9, 265.6)	703.4 (501.9, 985.7)	<0.001
acrylonitrile	0.77 (0.68, 0.89)	0.74 (0.65, 0.85)	0.69
benzene	3.3 (2.5, 4.5)	2.8 (2.1, 3.7)	0.38
carbon disulfide	2.3 (1.8, 3.0)	2.4 (1.8, 3.1)	0.81
dimethyl sulfide	1.7 (1.3, 2.3)	1.7 (1.3, 2.2)	0.92
ethanol	159.5 (117.5, 216.3)	115.3 (85.4, 155.7)	0.15
isoprene	18.2 (14.3, 23.3)	17.6 (13.8, 22.4)	0.84
pentane	17.5 (13.7, 22.4)	37.7 (29.6, 48.0)	<0.001
ammonia	71.4 (63.0, 81.1)	68.4 (60.4, 77.5)	0.64
hydrogen sulfide	0.39 (0.27, 0.57)	0.48 (0.33, 0.70)	0.48
triethyl amine	0.88 (0.64, 1.2)	0.90 (0.66, 1.2)	0.95
trimethyl amine (TMA)	10.2 (7.7, 13.5)	34.2 (26.4, 44.4)	<0.001

* Liver patients include patients with cirrhosis and alcoholic hepatitis and those with cirrhosis and acute decompensation.

Values presented as Mean (95% CL) and p-values obtained from Analysis of Covariance (ANCOVA). The natural logarithm of each compound was modeled as the outcome variable with group, gender and age as the independent variables.

A significance criterion of 0.004 (0.05/15) was used to account for multiple testing.

Values presented as Mean (95% CL) and p-values obtained from Analysis of Covariance (ANCOVA). The natural logarithm of each compound was modeled as the outcome variable with group, gender and age as the independent variables.

A significance criterion of 0.004 (0.05/15) was used to account for multiple testing.

Table-3

Breath compounds and the severity of liver disease in alcoholic hepatitis

Factor	MELD Score		Child's Pugh Score		Maddrey's Function	
	rho (95% CI)	p-value	rho (95% CI)	p-value	rho (95% CI)	p-value
2-propanol	-0.04 (-0.37,0.29)	0.81	0.01 (-0.32,0.35)	0.94	-0.06 (-0.39,0.27)	0.73
acetaldehyde	0.25 (-0.07,0.57)	0.13	0.10 (-0.24,0.43)	0.57	0.19 (-0.14,0.52)	0.25
acetone	0.29 (-0.03,0.61)	0.076	0.06 (-0.28,0.39)	0.73	0.23 (-0.10,0.55)	0.17
acrylonitrile	-0.27 (-0.59,0.05)	0.092	-0.12 (-0.46,0.22)	0.47	-0.28 (-0.60,0.04)	0.089
benzene	-0.00 (-0.34,0.33)	0.98	0.02 (-0.32,0.36)	0.89	-0.05 (-0.39,0.28)	0.74
carbon disulfide	0.13 (-0.20,0.46)	0.43	0.05 (-0.29,0.39)	0.77	0.05 (-0.29,0.38)	0.78
dimethyl sulfide	-0.10 (-0.43,0.24)	0.56	-0.04 (-0.37,0.30)	0.83	-0.03 (-0.36,0.31)	0.87
ethanol	0.18 (-0.15,0.51)	0.27	0.14 (-0.19,0.48)	0.4	0.18 (-0.15,0.51)	0.27
isoprene	0.13 (-0.20,0.46)	0.42	0.09 (-0.25,0.42)	0.6	0.12 (-0.21,0.45)	0.46
pentane	0.30 (-0.01,0.62)	0.06	0.12 (-0.21,0.46)	0.47	0.22 (-0.11,0.54)	0.19
ammonia	0.23 (-0.10,0.55)	0.17	0.11 (-0.23,0.44)	0.52	0.28 (-0.04,0.60)	0.09
hydrogen sulfide	0.15 (-0.18,0.48)	0.35	-0.02 (-0.36,0.31)	0.89	0.12 (-0.21,0.45)	0.48
triethylamine	-0.13 (-0.46,0.20)	0.43	0.01 (-0.33,0.35)	0.95	-0.06 (-0.39,0.27)	0.72
Trimethylamine (TMA)	0.38 (0.07,0.69)	0.018	0.17 (-0.16,0.50)	0.31	0.30 (-0.02,0.62)	0.062

MELD=Model for end-stage liver disease