Volatile organic compounds in bile can diagnose malignant biliary strictures in the setting of pancreatic cancer: a preliminary observation

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Background: Ascertaining the nature of biliary strictures is challenging. The role of volatile organic compounds (VOCs) in bile in determining the cause of biliary strictures is not known.

Objective: To identify potential VOCs in the headspaces (gas above the sample) of bile in patients with malignant biliary strictures from pancreatic cancer.

Design: Prospective cross-sectional study.

Setting: Referral center.

Patients: Prospective study in which bile was aspirated in 96 patients undergoing ERCP for benign and malignant conditions.

Main Outcome Measurements: Selected ion flow tube mass spectrometry (VOICE200R SIFT-MS instrument; Syft Technologies Ltd, Christchurch, New Zealand) was used to analyze the headspace and to build a predictive model for pancreatic cancer.

Results: The headspaces from 96 bile samples were analyzed, including 24 from patients with pancreatic cancer and 72 from patients with benign biliary conditions. The concentrations of 6 compounds (acetaldehyde, acetone, benzene, carbon disulfide, pentane, and trimethylamine [TMA]) were increased in patients with pancreatic cancer compared with controls ($P < .05$). By using receiver-operating characteristic curve analysis, we developed a model for the diagnosis of pancreatic cancer based on the levels of TMA, acetone, isoprene, dimethyl sulfide, and acetaldehyde. The model [10.94 + 1.8229* log (acetaldehyde) + 0.7600* log (acetone) − 1.1746* log (dimethyl sulfide) + 1.0901* log (isoprene) − 2.1401 * log (trimethylamine) ≥ 10] identified the patients with pancreatic cancer (area under the curve = 0.85), with 83.3% sensitivity and 81.9% specificity.

Limitations: Sample size.

Conclusions: The measurement of biliary fluid VOCs may help to distinguish malignant from benign biliary strictures. Further studies are warranted to validate these observations. (Clinical Trial Registration Number NCT01565460.) (Gastrointest Endosc 2014;80:1038-45.)

Abbreviations: AUC, area under the receiver-operating characteristics curve; ROC, receiver-operating characteristic; TMA, trimethylamine; VOC, volatile organic compound.

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Biliary strictures are challenging to diagnose and manage.[1] Determining whether the strictures are benign or malignant is important. Brush cytology performed during ERCP is the initial step in the diagnosis of biliary strictures.[1-3] However, the brushings are not always diagnostic, and their sensitivity varies from 20% to 50% in most studies.[4-7]

Pancreatic cancer is often diagnosed based on findings on radiological imaging, coupled with EUS with FNA and/or ERCP with brushings. However, in some patients without an obvious mass lesion on imaging or endoscopic studies, diagnosis may be challenging.[8,9]

Volatile organic compounds (VOCs) can be detected in the headspace of bile. Headspace is the gas space above the sample in a chromatography vial. Volatile sample components diffuse into the gas phase, forming the headspace gas. Headspace analysis is therefore the analysis of the components present in that gas. Headspace detects VOCs in bile by analyzing the gas above the bile sample. VOCs detected in bile are more likely to represent endogenously produced VOCs in certain disease processes such as cancer. Identification of potential biomarkers in the headspace of bile could aid in the diagnosis of cancer.[10]

The aim of our study was to identify potential VOCs in the bile that can help distinguish malignant biliary strictures in the setting of pancreatic cancer from benign strictures.

Take-home Message

- Measurement of volatile organic compounds (VOCs) from the headspace of bile is simple and feasible, but the role of VOCs in biliary fluid has not been studied.
- Measurement of VOCs in biliary fluid helps to distinguish pancreatic cancer–related malignant biliary stricture with 83.3% sensitivity and 81.9% specificity.

METHODS

Patients

The Cleveland Clinic biliary fluid database is a prospectively maintained database of bile obtained by direct aspiration from the common bile duct in patients referred to our center for ERCP. We established this database in 2012; it includes all patients who have bile aspirated before contrast injection at the time of ERCP at our center. The study was approved by the Cleveland Clinic Institutional Review Board and registered with the National Institutes of Health Clinical Trials registry.

Inclusion and exclusion criteria

The inclusion criteria were the ability to give informed consent and age older than 18 years. Patients who have acute cholangitis are not included in our biliary fluid database. The diagnosis of pancreatic cancer was based on tissue diagnosis either at surgery or on FNA on subsequent EUS on follow-up. Tissue diagnosis was established in all patients with pancreatic cancer in our cohort.

Biliary fluid sampling procedure

At the time of ERCP, once we cannulate the common bile duct, approximately 1 to 5 mL of bile is aspirated through the sphincterotome. We transport these bile samples to the laboratory on ice and freeze at −80°C until use.

VOC measurement in bile

Bile samples were centrifuged for 8 minutes at 150*g* and 4°C. The samples were heated to 40°C to allow the VOCs in the headspace to equilibrate with the samples. Twenty milliliters of headspace was removed from the sample with a gas syringe and analyzed with selected ion flow tube mass spectrometry (VOICE200 SIFT-MS instrument; Syft Technologies Ltd, Christchurch, New Zealand).[10] The mass spectrometry assay comprised the following 22 common analytes: 2-propanol, acetaldehyde, acetone, acetonitrile, acrylonitrile, benzene, carbon disulfide, dimethyl sulfide, ethanol, isoprene, pentane, 1-decene, 1-heptene, 1-nonene, 1-octene, 3-methylhexane, 2-nonene, ammonia, ethane, hydrogen sulfide, triethylamine, and trimethylamine (TMA).

The measurements were made in duplicate at the time of the analysis. The coefficient of variation for each VOC was within 5%.

Statistical analysis

Descriptive statistics were computed for all factors. These include medians, 25th and 75th percentiles, range or mean and standard deviation for quantitative variables, and frequencies and percentages for categorical factors. Variables were analyzed by using the *t* test and Wilcoxon rank sum test. Specifically, linear models of bile VOCs were created with pancreatic cancer and benign biliary conditions serving as independent variables. Logistic regression analysis was performed to build a predictive model for pancreatic cancer. The variables for the logistic regression models were selected in a stepwise fashion based on the greatest improvement among candidate predictors in the Akaike information criterion. Some variables selected were not significant in univariable analyses and may be partly attributed to some parameters having a stronger estimated association with diagnosis grouping (compared with univariable results) when adjusting for other parameters as covariates. Pancreatic cancer versus benign biliary conditions was modeled as the outcome, and the different compounds were considered for inclusion. Receiver-operating characteristic (ROC) analysis was performed, and the area under the ROC curves (AUC) and corresponding 95% confidence intervals were calculated. DeLong's method was used to compare the AUCs. R software version 2.15.2 (The R Foundation for