Research Article

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Use of the methacetin breath test to classify the risk of cirrhotic complications and mortality in patients evaluated/listed for liver transplantation

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Background & Aims: The MELD score predicts short-term mortality in patients with cirrhosis; however, some patients with low scores develop complications and die unexpectedly. Consequently, we evaluated the diagnostic accuracy of the methacetin breath test (MBT), an assay of liver metabolic function, and the MELD score, to predict the risk of complications of cirrhosis and liver-related death.

Methods: One hundred sixty-five patients with cirrhosis received oral ^{13}C-methacetin; $^{13}CO_2$ was measured in expired breath (BreathID®; Exalenz). The cumulative percent dose recovery of $^{13}CO_2$ at 20 min with a threshold of ⩽0.55% (high-risk) and >0.55% (low risk) most accurately predicted liver-related death and the risk of cirrhotic complications within one year. MELD thresholds of ⩾15 and ⩾19 were also examined to predict the same endpoints.

Results: Dose recovery ⩽0.55% and MELD ⩾19 both predicted liver-related death (HR 12.6 [95% CI 1.6–98.3]; $p = 0.016$, and HR 5.5 [1.6–18.9]; $p = 0.007$, respectively); MELD ⩾15 did not. Dose recovery ⩽0.55% (HR 1.9 [1.1–3.2]; $p = 0.03$) also predicted the risk of ⩾1 complication(s), and was particularly able to foretell the risk of development/exacerbation of ascites (HR 4.7 [1.8–11.9]; $p = 0.001$), which was not achieved by either MELD threshold. Finally, in patients with MELD <19, dose recovery ⩽0.55% predicted the risk of death ($p = 0.017$), development of ⩾1 cirrhotic complication(s) ($p = 0.062$), and development/exacerbation of ascites ($p = 0.0009$).

Conclusions: In this pilot study, methacetin breath testing predicted the risk of liver-related death and development/exacerbation of ascites more accurately than MELD ⩾15 or ⩾19. In patients with low MELD (<19 points), MBT may be useful to identify patients in whom the frequency of clinical observation should be intensified.

Keywords: Methacetin; Liver transplantation; Cirrhosis; MELD score.

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Abbreviations: MELD, Model for End-stage Liver Disease; UNOS, United Network for Organ Sharing; INR, International Normalized Ratio of the prothrombin time; LT, liver transplantation; MBT, methacetin breath test; HCC, hepatocellular carcinoma; CPDR, cumulative percent dose recovered; LRD, liver-related death; TIPS, transjugular intrahepatic porto-systemic shunt; PDR, percent dose recovery; SBP, spontaneous bacterial peritonitis; ROC, receiver operating characteristic; AUC, area under the ROC curve; HR, hazard ratio; CI, confidence interval; MEGX, monoethylglycinexylidide; HVPG, hepatic venous pressure gradient.

Introduction

In many nations including the US, the allocation of a liver graft from a deceased donor to a patient with end-stage liver disease follows the overriding dictum, transplant the sickest first. In 2002, the Model for End-Stage Liver Disease (MELD) score was widely adopted by the United Network for Organ Sharing (UNOS) and many other agencies as a tool for organ allocation as it accurately predicts three-month mortality, based on the prognostic information inherent in a patient's international normalized ratio (INR) of the prothrombin time, bilirubin, and creatinine [1]. Early analyses of UNOS data suggested that MELD was more accurate in predicting early mortality than Child-Pugh-Turcotte score as waitlist mortality in the US decreased [2,3], but deficiencies in MELD remain widely recognized, particularly in patients with low scores [4]. Although a series of MELD Exception Rules has been developed to compensate for specific complications of cirrhosis which increase 3-month mortality in patients with low MELD [5], some patients with low MELD scores continue to die while awaiting liver transplantation (LT).

Several studies have attempted to identify additional risk factors for mortality in patients with low MELD scores. For example, in US veterans with cirrhosis and low MELD, Heuman, *et al.* [6], first recognized the additional negative prognostic implications of persistent ascites and hyponatremia. Subsequent analyses demonstrated higher accuracy predicting death using the combination of MELD and serum sodium concentration, particularly in patients with low scores [7,8]. Another potential approach to assess prognosis in patients with mildly decompensated cirrhosis may be to quantify residual hepatic metabolic capacity with an

objective test of "true liver function". In this context, liver metabolic tests have been used for several decades in patients with acute and chronic liver disorders, and are based on measuring metabolites of ingested labeled substrates that are biotransformed exclusively by the liver. The amplitude and rate of appearance of the labeled metabolite represent liver enzymatic activity; hence, a decline may represent hepatic injury or decreased access of substrate to hepatocytes because of portosystemic shunting. Candidate methods include the indocyanine green clearance test [9], cholate shunt test [10], aminopyrine breath test [11], and methacetin breath test (MBT) [12].

MBT relies on the metabolic capacity of hepatic cytochrome P_{450} 1A2 to demethylate an ingested dose of ^{13}C-labeled methacetin into acetaminophen and ^{13}C-formaldehyde, and through further biotransformation, into $^{13}CO_2$, which can be detected in expired breath [13]. Although the MBT test is potentially useful, it has not been integrated into everyday clinical practice. A major drawback in using traditional breath tests is the cumbersome method of isotopic ratio mass spectrometry, which requires prolonged testing and analysis, and is not thereby suitable for point-of-care clinical use. The BreathID® continuous online ^{13}C-MBT (Exalenz Bioscience, Ltd.) is based on the measurement of exhaled breath $^{12}CO_2$ and $^{13}CO_2$ concentrations using correlation spectroscopy that can detect variations of less than 1/1000 in the $^{13}CO_2/^{12}CO_2$ ratio [12–14]. This rapid, point-of-care device is capable of continuous quantification of expired $^{13}CO_2$ collected via a nasal cannula. The results obtained from the device are expressed primarily as increases (delta) over baseline change in $^{13}CO_2/^{12}CO_2$ ratio from the breath sample prior to ingestion. Many time-dependent variables can be calculated from delta-over-baseline values.

MBT has been used to assess the functional capacity of the liver in several clinical conditions. In patients with hepatocellular carcinoma (HCC) undergoing major hepatectomy, the **c**umulative **p**ercentage of ^{13}C **d**ose **r**ecovered over time (CPDR) was shown to correlate closely with liver volume during regeneration as measured by computerized tomography ($r = 0.94$, $p < 0.001$), and was the only predictor of outcome, namely liver failure and/or mortality [15]. Another pilot study performed in patients with acute liver failure, has shown MBT to be useful in predicting transplant-free survival [14].

In the current study, we present data that explore a possible role for MBT to predict liver-related death (LRD) and other complications of cirrhosis in stable patients with advanced liver disease. The study population included patients undergoing evaluation, or listed, for LT in three study sites in the US and one in Israel. Subsequent to the performance of MBT, study patients were followed for one year or until death or LT. We first tested a set of variables derived from the raw delta-over-baseline data to identify the one most predictive of LRD and other complications of cirrhosis. We then compared the risk stratification of this MBT variable after selection of a cut-point, to two threshold MELD scores, focusing on patients with low MELD ($\geqslant$15 and $\geqslant$19), in whom death or life-threatening events would not be anticipated within the year of follow-up.

Methods

Patients

Patients were included in the study if they were >18 years old, listed for LT or undergoing evaluation for listing. One hundred sixty-five (n = 165) patients were recruited consecutively from the liver transplant programs of four centers: Virginia Commonwealth University (n = 76), Hadassah Medical University (n = 68), Medical University of South Carolina (n = 12), and Henry Ford Hospital (n = 9). All patients had biopsy-proven cirrhosis of any etiology, or had clinical evidence of cirrhosis (ascites and/or esophagogastric varices documented by endoscopy and/or hepatic encephalopathy), or laboratory evidence of cirrhosis by two of the following: platelet count of $<100 \times 10^9$/L in the absence of bone marrow disease, serum albumin <3.0 gm/dl, INR >1.5, and/or AST/ALT ratio of >1.0. Exclusion criteria included prior insertion of a transjugular intrahepatic portosystemic shunt (TIPS), known or suspected HCC, congestive heart failure (left-ventricular ejection fraction <20%), pulmonary hypertension (systolic pulmonary arterial pressure >45 mmHg on echocardiography), uncontrolled diabetes (hemoglobin A1c >9.5%), current treatment with any immunosuppressive medication, history of bariatric surgery or extensive small bowel resection, current receipt of parenteral nutrition, contraindication to oral medication, history of allergy to acetaminophen, pregnancy, current exacerbation of reactive airways disease/obstructive pulmonary disease, or patients taking potentially hepatotoxic drugs.

Approval for this study was obtained by the Institutional Review Boards at each study site, and informed consent was obtained from each patient meeting entry criteria. On the day of MBT, patients were instructed to fast for at least 8 h, avoid smoking on the day of the test, and avoid ethanol consumption or take drugs that are thought to interfere with methacetin metabolism (e.g., fluvoxamine, amiodarone, ciprofloxacin, cimetidine, rifampin, carbamazepine). Additionally, patients undergoing general anesthesia, receiving conscious sedation, or any acetaminophen-containing medications within the previous 24 h were excluded from the study. Seven patients were receiving a drug that could potentially interfere with cytochrome P450 1A2 activity (ciprofloxacin, famotidine or acyclovir), all of which have short half-lives (<4 h); therefore, patients were required a 24 h "wash-out" of these drugs prior to the breath test.

Study protocol

On the day of MBT determination, patients underwent a baseline medication review, physical examination and routine laboratory tests. MBT was determined using the BreathID® device according to the following procedure: After a 3–5 min rest period, a nasal cannula was placed on the patient, and baseline $^{13}CO_2/^{12}CO_2$ ratio was collected for 2 min. Patients then ingested 75 mg of ^{13}C-methacetin in 150 ml of water, and the patient's breath was collected and analyzed for an additional 60 min at rest in the seated position. As the test substrate is metabolized, the $^{13}CO_2/^{12}CO_2$ ratio changes and is presented in real time by the BreathID® device, which calculates the percentage dose recovery (PDR), expressed in %/hour, and the cumulative PDR, expressed in %/specified time period. Patients were seen at 3 month intervals in follow-up or as dictated by their clinical course. Interval histories, physical examinations, and medications were recorded with particular attention to any protocol-defined occurrence that signified deterioration related to cirrhosis: ascites (either the appearance or exacerbation of ascites, defined as the need for an increase in the dose of diuretics or for large-volume paracentesis), spontaneous bacterial peritonitis (SBP), gastrointestinal hemorrhage due to portal hypertension, hepatic encephalopathy, worsening in Child-Turcotte-Pugh score of $\geqslant$2 points on two or more consecutive visits, an increase in MELD score by $\geqslant$5 points on two or more consecutive visits, LT, and LRD.

Study participants were followed for one year from the time of first MBT or until a study endpoint was reached. For the 20 patients who died within this period, causes of death were assigned to one of three categories by site PI's: liver-related, liver-unrelated, or unclear. These assignments were reviewed by one investigator (Y.I.), who queried the site PI's to provide further details about unclear cases, and was blinded to the results of the MBT. After adjudication, 11 deaths were deemed liver-related, five liver-unrelated (progression of pancreatic carcinoma, perforation of gastric ulcer, pneumonia, renal failure, and sepsis in a renal transplant recipient), and four unclear.

MBT parameters were recorded electronically for each patient by the BreathID® device, including the PDR and CPDR at 5–10 min increments between 5 and 60 min, as well as the PDRpeak, time to peak, and CPDRpeak. For each parameter, a receiver operating characteristic (ROC) analysis was performed, and the area under the curve (AUC) calculated to obtain the most statistically robust differentiation between event-positive (i.e., an endpoint reached of LRD, LT, or complication of cirrhosis) and event-negative subjects. As shown in Supplementary Table 1, the AUC's for prediction of endpoints by the MBT parameters were very similar, with overlapping confidence intervals, except for the PDR and CPDR at 5 min. Therefore, we chose three parameters with higher AUC's (CPDR20, CPDR30 and CPDRpeak) for further study in Cox regression models to estimate the hazard ratios (HRs) for outcome variables. Supplementary Table 2 depicts HRs for LRD, LT, and complications of cirrhosis for CPDR20, CPDR30 and CPDRpeak. HRs <1 denote increased risk for lower breath test measurements.