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Quantitative Liver Function Tests Improve the Prediction of Clinical Outcomes in Chronic Hepatitis C: Results from the HALT-C Trial

Gregory T. Everson¹, Mitchell L. Shiffman², John C. Hoefs³, Timothy R. Morgan³, Richard K. Sterling⁴, David A. Wagner⁵, Shannon Lauriski¹, Teresa M. Curto⁶, Anne Stoddard⁶, Elizabeth C. Wright⁷, and the HALT-C Trial Group

Gregory T. Everson: greg.everson@ucdenver.edu; Mitchell L. Shiffman: Mitchell_Shiffman@bshsi.org; John C. Hoefs: jchoefs@uci.edu; Timothy R. Morgan: timothy.morgan@va.gov; Richard K. Sterling: RKSterli@hsc.vcu.edu; David A. Wagner: david@metsol.com; Shannon Lauriski: shannon.lauriski@ucdenver.edu; Teresa M. Curto: tcurto@neriscience.com; Anne Stoddard: astoddard@neriscience.com; Elizabeth C. Wright: wrightel@niddk.nih.gov

¹Section of Hepatology, Division of Gastroenterology and Hepatology, University of Colorado Denver, Aurora, CO.

²Liver Institute of Virginia, Bon Secours Health System, Newport News, VA.

³Division of Gastroenterology, University of California - Irvine, Irvine, CA and Gastroenterology Service, VA Long Beach Healthcare System, Long Beach, CA.

⁴Hepatology Section, Virginia Commonwealth University Medical Center, Richmond, VA.

⁵Metabolic Solutions, Inc., Nashua, NH.

⁶New England Research Institutes, Watertown, MA.

⁷Office of the Director, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Department of Health and Human Services, Bethesda, MD.

Abstract

Risk for future clinical outcomes is proportional to the severity of liver disease in patients with chronic hepatitis C. We measured disease severity by quantitative liver function tests (QLFTs) to

CONTACT INFORMATION, Gregory T. Everson, M.D., Professor of Medicine, Director of Hepatology, University of Colorado Denver, 1635 N Aurora Court, B-154, Aurora, CO 80045, Phone: 720-848-2245, FAX: 720-848-2246, greg.everson@ucdenver.edu.

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CONTRIBUTIONS OF AUTHORS

Gregory T. Everson, Mitchell L. Shiffman, John C. Hoefs, Timothy R. Morgan, and Richard K. Sterling were principal investigators at the participating clinical centers and were responsible for the overall conduct of the trial, review of data, presentation of results, and writing of this manuscript. David A. Wagner provided reagents and analysis of breath samples for the methionine breath test. Shannon Lauriski was the principal laboratory researcher who analyzed samples for cholate isotopes, caffeine, antipyrine, and MEGX. Teresa Curto, Anne Stoddard, and Elizabeth C. Wright provided the statistical support and analyses of data and participated in the writing and review of the manuscript.

FINANCIAL DISCLOSURES

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D.A. Wagner is employed, has equity, and has intellectual property rights with Metabolic Solutions.

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determine cutoffs for QLFTs that identified patients who were at low and high risk for a clinical outcome. Two hundred twenty seven participants in the Hepatitis C Antiviral Long-Term Treatment Against Cirrhosis (HALT-C) Trial underwent baseline QLFTs and were followed for a median of 5.5 years for clinical outcomes. QLFTs were repeated in 196 patients at month 24 and in 165 patients at month 48. Caffeine elimination rate (k), antipyrine (AP) clearance (Cl), MEGX concentration, methionine breath test (MBT), galactose elimination capacity (GEC), dual cholate (CA) clearances and shunt, and perfused hepatic mass (PHM) and liver and spleen volumes (SPECT) were measured. Baseline QLFTs were significantly worse ($p=0.0017$ to <0.0001) and spleen volumes larger ($p<0.0001$) in the 54 patients who subsequently experienced clinical outcomes. QLFT cutoffs that characterized patients as “low” and “high risk” for clinical outcome yielded hazard ratios ranging from 2.21 (95%CI 1.29–3.78) for GEC to 6.52 (95%CI 3.63–11.71) for CA Cl_{oral}. QLFTs independently predicted outcome in models with Ishak fibrosis score, platelet count, and standard laboratory tests. In serial studies, patients with “high risk” results for CA Cl_{oral} or PHM had a nearly 15-fold increase in risk for clinical outcome. Less than 5% of patients with “low risk” QLFTs experienced a clinical outcome.

Conclusion—QLFTs independently predict risk for future clinical outcomes. By improving risk assessment, QLFTs could enhance noninvasive monitoring, counseling, and management of patients with chronic hepatitis C.

Keywords

cholate; SPECT liver-spleen scan; methionine; caffeine; antipyrine; galactose; MEGX

Chronic hepatitis C is a major cause of liver disease, cirrhosis and hepatocellular carcinoma (HCC) in the United States and worldwide (1–4). Early detection of patients with significant hepatic impairment, who are at risk for future decompensation, is a priority of clinical management.

Progression of liver disease is defined histologically by accumulation of fibrosis and physiologically by impairment of hepatic function and blood flow. Increased Ishak fibrosis score (5,6) or increased hepatic venous pressure gradient (HVPG) (7–9) indicate greater severity of liver disease and identify patients at risk for future clinical complications. Quantifying fibrosis requires performance of liver biopsy and measuring HVPG is technically complex and requires catheterization of the jugular vein. Both liver biopsy and HVPG measurement are associated with potentially severe complications, prone to sampling error, and may not be embraced by patients. Accurate noninvasive methods for staging disease are needed.

One noninvasive approach is to develop models based on clinical findings and standard blood tests. Child-Turcotte-Pugh (CTP) classification (10) and model for end-stage liver disease (MELD) score (11,12), are perhaps the best known and most commonly applied. Both were developed to predict surgical mortality or mortality after transjugular intrahepatic portal-systemic shunt (TIPS) in patients with advanced cirrhosis. Neither are applicable to the patient with earlier-stage or clinically-compensated disease (13).

Other models target patients with compensated disease. The Hepatitis C Antiviral Long-term Treatment against Cirrhosis (HALT-C) Trial investigators developed a model based upon bilirubin, albumin, AST:ALT, and platelet count (14). This model identified high risk patients, 59% of whom developed clinical outcomes in 3.5 years of followup. But, the high risk cutoff was insensitive; only 46% of the patients who eventually developed outcomes were identified.

Hepatic elastography and serum fibrosis markers correlate with stage of fibrosis, and risk for cirrhosis or varices (15–18). In one study, hyaluronic acid, YKL-40, and TIMP-1 combined with standard laboratory tests were significantly associated with disease progression (18). Further studies of elastography and serum fibrosis markers in predicting future risk for clinical outcomes are needed to validate their prognostic value.

We have previously demonstrated that a battery of QLFTs correlated with stage of fibrosis, risk for cirrhosis and varices, size of varices, and other indicators of disease severity in patients enrolled in the HALT-C Trial (19,20). In the current study, we evaluated the independent ability of these QLFTs to prospectively define the risk for development of future clinical outcomes (hepatic decompensation or liver-related death).

PATIENTS AND METHODS

The designs and methods of the HALT-C trial and the QLFT ancillary study have been previously described (19–21). All patients had advanced fibrosis or cirrhosis and had previously failed to achieve sustained virologic response (SVR) with a prior course of interferon or peginterferon with or without ribavirin. Most importantly, no patient had a prior history of any clinical complication of liver disease and all had baseline CTP scores of 5 or 6. Three clinical centers enrolled patients – University of Colorado Denver, Virginia Commonwealth University, and University of California, Irvine.

Baseline QLFTs were performed in 285 patients. “Lead-In” patients (n=232) underwent baseline QLFTs prior to retreatment with peginterferon and ribavirin in the Lead-In Phase of HALT-C. “Express” patients (n=53) were treated with peginterferon plus ribavirin prior to enrollment in HALT-C and underwent baseline QLFTs just prior to randomization. Thirty two “Lead-In” patients who achieved SVR, 9 relapsers and 7 nonresponders did not participate in the randomized phase, and 10 dropped from study before week 20. The remaining 227 patients (174 “Lead-In” and 53 “Express”) formed the cohort for the current study and were randomized to untreated control (n=120) or maintenance with low dose peginterferon monotherapy (n=107). Patients were followed for clinical outcomes for a median of 5.5 yr, mean of 4.9 ± 2.2 yr, and range from 0 to 8.3 yr. QLFTs were repeated at month 24 in 196 patients and at month 48 in 165 patients.

Assessment of clinical outcomes

Patients were evaluated every 3 months during the period of follow-up. Clinical outcomes included CTP progression (CTP score ≥ 7 on two consecutive evaluations), variceal bleeding, ascites, hepatic encephalopathy, and liver-related death. Listing for liver transplantation, liver transplantation, HCC, presumed HCC, and death due to non-hepatic causes were not outcomes in this analysis. Ten patients underwent liver transplantation, four for presumed HCC, and six for hepatic decompensation. In these six patients liver transplantation occurred subsequent to a different initial clinical outcome (CTP progression in 4 and encephalopathy in 2). The 4 patients with liver transplantation prior to clinical outcome were included in our analyses but censored at the time of transplantation. An Outcomes Review Panel, comprised of investigators from three clinical centers of the HALT-C Trial, verified all outcomes (21).

Statistical Analyses

Results are expressed as means, standard deviations, and ranges. Baseline differences in demographic, clinical, histologic, and endoscopic characteristics, and results of QLFTs between patients with and without clinical outcomes were evaluated by Cox proportional hazards analysis.

QLFT results were divided into tertiles of equal numbers of patients, stratifying results into low, intermediate, or high ranges, and the risks for clinical outcomes across QLFT tertiles were analyzed by Kaplan-Meier log-rank tests. QLFT cutoffs were defined using the boundary for the high risk tertile and these cutoffs were further verified by ROC (receiver operator curve) analyses. The independence of QLFTs in predicting clinical outcomes was analyzed by multivariable models that included histologic stage (Ishak fibrosis scores 2,3,4 versus 5,6) and platelet count or the HALT-C laboratory model (14). The performance of these same QLFT cutoffs in predicting initial clinical outcome was also evaluated in the serial QLFT studies by pooled relative risk analyses (Mantel-Haenzel method). In the latter analyses patients were censored once they had experienced a clinical outcome.

Statistical analyses were performed at the Data Coordinating Center for HALT-C (New England Research Institutes) using SAS release 9.2 (SAS Institute, Cary, NC).

RESULTS

Clinical Outcomes

Fifty four patients (24%) experienced at least one clinical outcome. These included progression in the CTP score (N=37), variceal bleeding (N=4), ascites (N=4), hepatic encephalopathy (N=6), and liver-related death (N=3). Nineteen patients whose initial outcome was an increase in CTP score subsequently experienced 28 additional clinical outcomes (ascites (n=13), liver-related death (n=10), encephalopathy (n=4), and spontaneous bacterial peritonitis (n=1)). Clinical outcomes occurred in 12% of patients with Ishak fibrosis scores of 3 or 4 and in 40% of patients with Ishak fibrosis scores of 5 or 6.

Lack of Treatment Effect on QLFTs

In the main HALT-C Trial peginterferon alfa-2a, 90 µg/wk, failed to improve clinical outcomes or halt histologic progression (22). In the current study untreated patients and patients treated with maintenance peginterferon had similar baseline QLFTs and treatment had no effect on the changes in QLFTs from baseline to months 24 and 48 (Table 1). Given the lack of treatment effect, treatment and control groups were combined for the analyses of QLFTs in predicting clinical outcomes.

Baseline Demographic and Clinical Variables Associated with Clinical Outcomes

Baseline patient characteristics and standard laboratory results of patients with and without subsequent clinical outcomes are listed in Table 2. Patients who developed outcomes had higher bilirubin and INR, and lower albumin. Although these differences were statistically significant, the means for these tests were within the normal range, even in the patients who developed outcomes. Only 6% of the patients who developed outcomes had INR>1.2, 22% had bilirubin >1.2 mg/dL, and 52% had albumin <3.5 g/dL. In contrast, the mean platelet count of patients who developed outcomes was below the lower limit of the normal range and 70% had a platelet count <140,000/µL.

Patients with subsequent outcomes had higher fibrosis scores, and were more likely to have cirrhosis on liver histology and varices at endoscopy.

Baseline QLFTs Associated with Clinical Outcomes

QLFTs were worse at baseline in the patients who subsequently experienced clinical outcomes (Table 2). Although differences varied by test, patients who in followup had subsequent clinical outcomes had greater hepatic impairment, including microsomal (antipyrine, caffeine, and lidocaine-MEGX), mitochondrial (methionine), and cytosolic

(galactose) functions and flow-dependent clearances ((galactose, cholates, perfused hepatic mass)).

QLFTs are more sensitive than standard liver blood tests in identifying the patients with hepatic impairment. In contrast to standard laboratory tests, baseline QLFTs were beyond the normal range in nearly all of the patients who developed outcomes. Sixty four percent had caffeine elimination rate (k_{elim}) $<0.05 \text{ h}^{-1}$, 89% had antipyrine (AP) $k_{elim} <0.04 \text{ h}^{-1}$, 80% had AP clearance (Cl) $<0.4 \text{ mL min}^{-1} \text{ kg}^{-1}$, 81% had monoethylglycylxylidide concentration at 15 minutes post lidocaine (MEGX_{15min}) $<20 \text{ ng/mL}$, 73% had methionine breath test (MBT) <50 , 74% had galactose elimination capacity (GEC) $<5 \text{ mg min}^{-1} \text{ kg}^{-1}$, 93% had cholate (CA) clearance after oral administration (Cl_{oral}) $<15 \text{ mL min}^{-1} \text{ kg}^{-1}$, 89% had CA shunt $>30\%$, and 79% had perfused hepatic mass (PHM) <100 .

Predicting Clinical Outcomes by QLFTs

Figure 1 shows the relationships of tertiles of baseline metabolic QLFTs to subsequent development of clinical outcomes. Methionine breath test and antipyrine clearance performed best. The boundaries and hazard ratios (HR) for high risk tertiles, which also defined QLFT cutoffs, were MBT ≤ 48 (HR 5.92), AP Cl $\leq 0.28 \text{ mL kg}^{-1} \text{ min}^{-1}$ (HR 3.62), caffeine $k_{elim} \leq 0.04 \text{ h}^{-1}$ (HR 2.67), MEGX_{15min} $\leq 9.0 \text{ ng/mL}$ (HR 2.50), and GEC $\leq 4.32 \text{ mg kg}^{-1} \text{ min}^{-1}$ (HR 2.21) (Table 3). By ROC analyses, the c-statistic for MBT was 0.79.

Figure 2 shows the relationships of tertiles of baseline cholate clearances and shunt and SPECT liver-spleen scan results to subsequent clinical outcomes. The boundaries and HRs for high risk tertiles were CA Cl_{oral} $\leq 9.47 \text{ mL kg}^{-1} \text{ min}^{-1}$ (HR 6.52), PHM ≤ 94.5 (HR 4.97), spleen volume $\geq 5.93 \text{ mL kg}^{-1}$ (HR 4.16), and CA Shunt $\geq 46\%$ (HR 3.98) (Table 3). By ROC analyses, c statistics were 0.84 for CA Cl_{oral}, 0.79 for cholate shunt, 0.79 for PHM, and 0.78 for spleen volume.

Baseline prevalence of cirrhosis (Ishak fibrosis stage 5 or 6) was higher and platelet count lower in the patients who subsequently experienced clinical outcomes (Table 2). Therefore, we tested the independence of QLFTs in predicting clinical outcomes by including these two factors as covariates. Interestingly, histologic stage dropped from significance in the prediction of clinical outcomes in models with AP Cl, CA Cl_{oral}, CA Shunt, PHM, and spleen volume. Each QLFT, except spleen volume, retained significance in predicting clinical outcome in models of the QLFT with platelet count and histologic stage (Table 3).

We further tested the independence of QLFTs in models of each QLFT with the HALT-C laboratory score which is derived from platelet count, bilirubin, albumin, and AST:ALT ratio. MBT, CA Cl_{oral}, PHM, and spleen volume remained significant and cholate shunt approached significance in these models (Table 3).

Serial QLFTs and Clinical Outcomes

Figure 3 displays the results for the serial QLFTs. The percentages of patients above and below QLFT cutoffs who experienced clinical outcomes during the two year intervals following QLFT studies at baseline, month 24, and month 48 are shown. AP Cl, caffeine k_{elim} , CA Cl_{oral}, CA Shunt, PHM, and spleen volume performed best. Eleven to 30 percent of patients characterized as high risk by QLFTs experienced their initial clinical outcomes in the 2 year intervals between testing periods. Pooled relative risks (RR) for initial clinical outcomes, based on these QLFT cutoffs were (RR (95%CI)) AP Cl 7.25 (2.98–17.63), caffeine k_{elim} 5.63 (2.66–11.90), GEC 3.08 (1.73–5.49), MEGX_{15min} 2.48 (1.33–4.61), MBT 5.43 (2.18–13.55), CA shunt 7.62 (3.77–15.42), CA Cl_{oral} 14.09 (6.03–32.95), PHM 14.47 (6.24–33.55), spleen volume 6.07 (3.10–11.89). Sensitivities (pooled) of the serial QLFTs in identifying the patients who developed outcomes were: CA Cl_{oral} 86%, PHM

83%, AP Cl 80%, cholate shunt 79%, caffeine k_{elim} 76%, MBT 75%, spleen volume 72%, GEC 57%, and MEGX_{15min} 51%.

Perhaps even more importantly, characterization of a patient as “low risk” by QLFT cutoffs was associated with a very low risk for clinical outcome. The negative predictive values (pooled) for clinical outcome of QLFT cutoffs defining “low risk” were: CA Cl_{oral} 98.4%, PHM 98.2%, AP Cl 97.6%, cholate shunt 97.6%, caffeine k_{elim} 97.1%, MBT 97.4%, spleen volume 97.0%, GEC 95.3%, and MEGX_{15min} 95.0%.

At each testing period, the mean values for QLFTs (except GEC) were significantly worse in the group of patients experiencing subsequent clinical outcomes. In addition, in the patients whose initial clinical outcome occurred after the month 48 QLFT study, mean values for nearly all QLFTs worsened from either baseline or month 24 to month 48 (data not shown).

DISCUSSION

One goal of our study was to define the impact of peginterferon maintenance therapy on hepatic function in patients with advanced but compensated chronic hepatitis C. In three large clinical trials, maintenance therapy failed to slow disease progression or reduce clinical outcomes (22–24). In the HALT-C Trial, serum HCV RNA and ALT and hepatic inflammation were reduced by maintenance therapy (22). The latter effects could potentially reflect reduction in hepatic injury, which might improve hepatic function or blood flow. However, in the current study peginterferon maintenance therapy failed to improve any of the serially performed QLFTs – a group of tests that evaluated a broad range of hepatic functions and blood flow. The lack of improvement in QLFTs in the current study provides additional evidence that maintenance therapy with low dose peginterferon is ineffective.

Another major goal of our study was to evaluate the independent ability of QLFTs to predict future clinical outcomes. Our patient cohort was ideal for addressing this goal because all had advanced fibrosis, all were at-risk for future clinical outcome, and none had experienced prior decompensation. In followup, 24% of our cohort experienced a clinical outcome which was similar to the rate of clinical outcome observed in the HALT-C Trial as a whole (22). Our results are likely representative of the whole HALT-C cohort and the general population of, compensated patients with advanced chronic hepatitis C.

Because QLFTs monitor changes in hepatic metabolism and blood flow, changes which are common to all liver diseases, they could potentially be useful in monitoring patients with any liver disease. Despite a 48.5% prevalence of hepatic steatosis in our cohort, the relationships of cholate clearances, shunt, and perfused hepatic mass to stages of hepatic fibrosis and cirrhosis are preserved and not altered by BMI, hepatic steatosis, HOMA score, hepatic inflammation, alcohol use, and smoking (19). In addition, SPECT liver-spleen scan has correlated with the severity of a variety of liver diseases (25–28).

Progression of chronic hepatitis C is characterized pathologically by accumulation of fibrosis and physiologically by impairment of hepatic function and blood flow. In our study, we measured physiologic impairment using a battery of QLFTs. We previously demonstrated that these QLFTs predicted cirrhosis, stage of fibrosis, varices, and variceal size (19). Also, they identified the subgroup of patients with most severe disease who failed to respond to antiviral therapy, and tracked improvement in hepatic function after sustained virologic response (20). In the current study QLFTs identified patients with greatest hepatic impairment who developed clinical outcomes.

We defined cutoffs for QLFTs that predicted risk for future clinical decompensation over a median duration of followup of 5.5 years. In multivariable analyses, all QLFTs enhanced

prediction of clinical outcomes when these tests were combined with hepatic histology and platelet count. In models with AP Cl, CA Cl_{oral}, CA shunt, PHM, and spleen volume, histologic cirrhosis dropped from significance. Cirrhosis did not improve the prediction of clinical outcomes by these QLFTs. In a prior analysis, CA Cl_{oral}, CA shunt, and PHM were able to predict which patients had varices – a prediction that was also not improved by adding hepatic histology to the models (19). These results raise the possibility that measurement of hepatic function by noninvasive QLFTs could be clinically relevant and useful and potentially supplant staging of hepatic fibrosis by liver biopsy as the “gold standard” for defining risk for future outcomes. Our results also suggest that QLFTs could complement histology and standard laboratory tests in the assessment of a patient’s risk for hepatic decompensation and liver-related death.

Serial testing identified high-risk patients from our initial cohort of stable patients with advanced fibrosis and compensated cirrhosis. The relative risk for clinical outcome was nearly 15-fold greater for patients with “high risk” compared to “low risk” results for CA Cl_{oral} and PHM. Serial QLFT testing identified up to 86% of all the patients who developed outcomes. Perhaps more importantly, less than 5% of patients with “low-risk” QLFT results experienced a clinical outcome. These findings indicate that serial QLFTs performed every two years can be useful in detecting not only the patients at highest risk for clinical outcome, but also the patients with stable disease who will have a benign clinical course.

Stage of fibrosis, especially histologic cirrhosis, determined by liver biopsy is considered the “gold standard” for assessing disease severity and predicting clinical outcome. In the HALT-C cohort with 6 years of followup, baseline Ishak fibrosis stage 6 (definite cirrhosis) or stages 5 (incomplete cirrhosis) plus 6 had 35% (83/238) and 66% (157/238) sensitivity for prediction of future clinical outcome (7). In the current study, serial QLFT testing was up to 86% sensitive. In the same study of histology, 18% (155/853) of patients with Ishak fibrosis stage <6 and 13% (81/622) of patients with Ishak fibrosis stage <5 experienced clinical outcomes (7). As noted above, <5% of patients with “low risk” QLFT results experienced a clinical outcome. These comparisons suggest that QLFTs may be superior to histologic staging by liver biopsy in identifying both high and low risk groups and more accurate than staging fibrosis (6,7,29–40) in predicting clinical outcomes.

Prognostic models utilizing standard blood tests (AST:ALT, bilirubin, albumin, platelet count) and Ishak fibrosis score were previously developed in the HALT-C cohort (14). However, we observed that the mean values for baseline bilirubin, INR, and albumin were within the normal range in the patients who subsequently developed clinical outcomes. In clinic populations with less severe disease the ability of these standard tests to identify the patients at higher risk for clinical outcomes would be limited. In addition, hepatic histology was a significant predictor of clinical outcome in these laboratory models, indicating that liver biopsy would still be required to optimize the prediction of risk for developing a clinical outcome. In contrast, nearly all of the patients who experienced clinical outcomes had values for QLFTs outside the normal range, suggesting that QLFTs could provide greater discrimination between high and low risk patients in clinic populations that are enriched with patients who have milder disease. Indeed, in the current study CA Cl_{oral}, PHM, spleen volume, MBT, and possibly cholate shunt enhanced the predictability of the HALT-C laboratory model.

Although normal ALT and minimal fibrosis on liver biopsy may imply minimal disease, a proportion of these patients have more advanced disease and are at risk for clinical outcomes (41–43). QLFTs could potentially be useful in this population by defining those with significant hepatic impairment who would be predicted to experience future clinical outcomes.

Historically, the clinical assessment of the patient with liver disease has relied upon surrogates of hepatic function (fibrosis stage or liver blood tests) as opposed to a true measurement of function. In the evaluation of disease affecting other organs, functional assessment defines prognosis and clinical management. Because fibrosis and standard blood tests have been the standards for assessing severity of liver disease, functional tests have been compared to these surrogates. Unfortunately, these comparisons cannot differentiate the advantages of functional testing over surrogates, or vice versa. Using a relevant and discriminating endpoint, clinical outcome, we compared QLFTs to hepatic histology and standard blood tests and demonstrated that QLFTs compared favorably to hepatic histology and enhanced standard blood tests in the prediction of clinical outcome.

Analyses of our battery of QLFTs suggests that cholate clearance and perfused hepatic mass performed best in identifying the patients at risk for clinical outcomes. When used serially these tests had the highest pooled relative risk, sensitivity, and negative predictive value. In contrast to cholate clearance and perfused hepatic mass, metabolic tests may be influenced by age, gender, medications, BMI, and hepatic steatosis (19,44–50).

We conclude that QLFTs identify the patients who are at risk for future clinical decompensation, and also the patients with adequate hepatic reserve who will have a benign clinical course. Despite these favorable characteristics, questions remain. Are QLFTs practical or ready for routine use in clinical practice, or, will any of the QLFTs gain approval by the US Food and Drug Administration? It is our opinion that broader clinical application of QLFTs is not only possible but likely. Noninvasive quantification of hepatic function and reserve by QLFTs is safer than determination of hepatic fibrosis by liver biopsy and QLFT methods have been simplified (51,52). Herein we demonstrated that QLFTs, particularly cholate clearance and perfused hepatic mass, more accurately predict risk for clinical outcome. Improved safety and accuracy are appealing to patients, care providers, regulatory bodies, and payors. Although elastography or serum fibrosis tests are safer than liver biopsy, they yield no additional characterization of liver disease beyond stage of fibrosis. In addition, elastography is expensive, operator dependent, and results may be influenced by body habitus, hepatic steatosis, and hepatic inflammation. We speculate that the time may come when quantifying liver function, in preference to measuring liver fibrosis, becomes the new standard for assessing disease severity in patients with chronic liver disease.

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LIST OF ABBREVIATIONS

PEG	peginterferon
QLFTs	quantitative liver function tests
HALT-C	Hepatitis C Antiviral Long-term Treatment against Cirrhosis
Cl	clearance
PHM	perfused hepatic mass
HCV	hepatitis C virus
GCRC	General Clinical Research Center
SD	standard deviation
INR	prothrombin time international normalized ratio
BMI	body mass index
IND	Investigational New Drug
MEGX	monoethylglycine xylidide
MBT	methionine breath test
SPECT	single photon emission computed tomography
k_{elim}	elimination rate constant
GEC	galactose elimination capacity
CTP	Child-Turcotte-Pugh
AST	aspartate aminotransferase
ALT	alanine aminotransferase

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Antipyrine CI

Methionine Breath Test

Caffeine k_{elim}

MEGX_{15min}

Galactose Elimination Capacity

Figure 1.

Metabolic tests. Results were divided into tertiles of equal numbers of patients, stratifying results into low (solid line), intermediate (dashed line), or high ranges (dotted and dashed line). Risks for clinical outcomes across tertiles were analyzed by Kaplan-Meier log-rank tests. Cutoffs were defined using the boundary for the high risk tertile. For these metabolic tests the high risk tertile had the lowest test results. Survival Probability is freedom from clinical outcome.

Cholate Cl_{oral}

Cholate Shunt

Cholate Cl_{iv}

Perfused Hepatic Mass

Spleen Volume by SPECT

Liver Volume by SPECT

Figure 2.

Cholate (CA) tests and SPECT liver-spleen scan results. Results were divided into tertiles of equal numbers of patients, stratifying results into low (solid line), intermediate (dashed line), or high ranges (dotted and dashed line). Risks for clinical outcomes across tertiles were analyzed by Kaplan-Meier log-rank tests. Cutoffs were defined using the boundary for the high risk tertile. For CA Cl_{iv} , CA Cl_{oral} , and PHM the high risk tertile had the lowest test results. For cholate shunt and spleen volume the high risk tertile had the highest test results. Survival Probability is freedom from clinical outcome.

Panel A

% Experiencing Clinical Outcome between Years 0 to 2

Panel B

% Experiencing Clinical Outcome between Years 2 to 4

Panel C

% Experiencing Clinical Outcome beyond Yr 4

Yr 4 QLFTs

Figure 3.

Incidence of clinical outcomes in 2 year intervals during serial studies. QLFT cutoffs were determined by prior Kaplan-Meier log-rank tests and ROC analyses. QLFT cutoffs defined two groups of patients, those at high versus low risk for clinical outcome. Patients with high risk QLFT results had an 11 to 30% chance of experiencing clinical outcome within 2 years. Patients with low risk QLFT results had a benign clinical course.

Table 1

Change of QLFTs from baseline comparing treated patients to controls

Variable	M24 - Base				M48 - Base					
	N	Treated	N	Control	p	N	Treated	N	Control	p
Metabolic Tests										
Antipyrine k_{elim} (h^{-1})	60	0 (0.013)	61	-0.002 (0.01)	0.49	53	0.001 (0.026)	46	0.001 (0.013)	0.96
Antipyrine Cl (mL min^{-1})	59	-0.97 (11.7)	59	-1.46 (9.05)	0.80	53	-3.34 (15.21)	44	-1.64 (10.06)	0.53
Caffeine k_{elim} (h^{-1})	78	-0.01 (0.046)	87	0.004 (0.058)	0.11	72	-0.018 (0.061)	67	-0.004 (0.037)	0.09
GEC (mg $kg^{-1} min^{-1}$)	93	0.265 (0.908)	98	-0.106 (0.913)	0.01	84	-0.225 (1.337)	79	-0.33 (0.993)	0.57
MEGX _{15min} (ng mL ⁻¹)	83	-1.98 (15.34)	93	-0.95 (14.68)	0.65	74	-5.74 (15.05)	74	-1.49 (16.43)	0.10
MBT	77	-5.71 (29.63)	80	-15.76 (37.14)	0.06	0		0		
Cholate Clearances and Shunt										
CA k_{elim} (min^{-1})	95	-0.003 (0.03)	99	-0.007 (0.039)	0.46	85	-0.002 (0.04)	79	-0.009 (0.041)	0.30
CA Cl _{iv} (mL min^{-1})	95	-15.7 (134.8)	99	-47.4 (124.2)	0.09	85	20.3 (158.0)	79	0.8 (137.9)	0.40
CA Cl _{oral} (mL min^{-1})	94	-18.5 (488.9)	99	-56.9 (395.7)	0.55	85	115.6 (498.2)	79	69.4 (717.2)	0.63
CA Shunt	94	0.02 (0.16)	99	-0.01 (0.17)	0.32	85	0.01 (0.17)	79	0.01 (0.17)	0.95
SPECT Liver-Spleen Scan										
PHM	89	0.49 (7.72)	92	-0.13 (6.17)	0.55	77	-0.74 (10.07)	72	-0.34 (6.83)	0.78
Liver Volume (mL kg^{-1})	89	-0.55 (2.78)	93	-1.04 (3.48)	0.30	76	-0.29 (3.34)	73	-0.92 (3.78)	0.29
Spleen Volume (mL kg^{-1})	88	0.39 (1.82)	92	0.18 (1.42)	0.39	76	0.80 (2.16)	72	0.69 (1.84)	0.73

Abbreviations: k_{elim} , rate constant of elimination; Cl, clearance; GEC, galactose elimination capacity; MEGX_{15min}, monoethylglycylglycidide concentration at 15 minutes after intravenous dose of lidocaine; MBT, methionine breath test; CA k_{elim} , rate constant of the rapid first phase of elimination of intravenously administered [24-¹³C]cholate; CA Shunt, equivalent to CA Cl_{iv}/CA Cl_{oral}; CA Cl_{iv}, clearance of intravenously administered [24-¹³C]cholate calculated from dose/AUC; CA Cl_{oral}, clearance of orally administered [2,2,4,4-²H]cholate calculated from dose/AUC; CA Shunt, calculated from CA Cl_{iv}/CA Cl_{oral}; PHM, perfused hepatic mass.

Table 2

Baseline assessment of patients with and without clinical outcomes

Variable	Patients without Outcomes			Patients with Outcomes			Cox Regression p value
	N	Mean/%	Std Dev	N	Mean/%	Std Dev	
Standard Tests and Histology							
Bilirubin (mg/dL)	173	0.7	0.31	54	0.95	0.53	<0.0001
Prothrombin, INR	173	1.01	0.09	54	1.08	0.12	<0.0001
Albumin (g/dL)	173	3.87	0.36	54	3.49	0.37	<0.0001
Platelets (1000 mm3)	173	176.2	63.5	54	123.4	67	<0.0001
Ishak Fibrosis Score	173	3.92	1.26	54	5.07	1.01	<0.0001
Cirrhosis on biopsy (Ishak 5–6)	173	34%		54	72%		<0.0001
Metabolic Tests							
Antipyrine k_{elim} (h ⁻¹)	114	0.036	0.015	36	0.027	0.01	0.0008
Antipyrine Cl (mL kg ⁻¹ min ⁻¹)	111	0.399	0.182	35	0.285	0.12	0.0004
Caffeine k_{elim} (h ⁻¹)	157	0.071	0.047	50	0.047	0.04	0.0008 ^a
GEC (mg kg ⁻¹ min ⁻¹)	170	4.95	1.2	53	4.39	1.11	0.002
MEGX _{15min} (ng mL ⁻¹)	169	19.5	13.7	48	12.5	11.1	0.002
MBT	145	71.8	37.3	40	42.1	23.1	<0.0001
Cholate Clearances and Shunt							
CA k_{elim} (min ⁻¹)	171	0.098	0.029	54	0.081	0.03	<0.0001
CA Cl _{iv} (mL kg ⁻¹ min ⁻¹)	170	4.65	1.47	54	3.71	1.47	<0.0001
CA Cl _{oral} (mL kg ⁻¹ min ⁻¹)	170	14.19	5.88	54	8.03	4.14	<0.0001
CA Shunt	171	0.363	0.137	54	0.522	0.17	<0.0001
SPECT Liver-Spleen Scan							
PHM	170	99.5	7.03	53	89.94	10.32	<0.0001
Liver Volume (mL kg ⁻¹)	169	18.52	3.41	53	18.82	3.52	0.50
Spleen Volume (mL kg ⁻¹)	167	4.33	2.28	52	7.31	3.84	<0.0001

Abbreviations: k_{elim} , rate constant of elimination; V_d, volume of distribution; Cl, clearance; GEC, galactose elimination capacity; MEGX_{15min}, monoethylglycylglycidide concentration at 15 minutes after intravenous dose of lidocaine;

MBT, methionine breath test; CA kelim, rate constant of the rapid first phase of elimination of intravenously administered $[24-^{13}\text{C}]$ cholate; CA Shunt, equivalent to $\text{CA Cl}_{\text{IV}}/\text{CA Cl}_{\text{oral}}$; CA Cl_{IV} , clearance of intravenously administered $[24-^{13}\text{C}]$ cholate calculated from dose/AUC; CA Cl_{oral} , clearance of orally administered $[2,2,4,4-^2\text{H}]$ cholate calculated from dose/AUC; CA Shunt, calculated from $\text{CA Cl}_{\text{IV}}/\text{CA Cl}_{\text{oral}}$; PHM, perfused hepatic mass.

^aThe p value for caffeine kelim was <0.0001 after adjustment for current smoking.

Table 3
Performance Characteristics of QLFT Cutoffs in Prediction of Clinical Outcomes

Category	QLFT	Tested	Patients (N)	High Risk QLFT Cutoff	Univariate HR (95% CI)	Multivariate (Histology, Platelet Count) HR (95% CI)	Multivariate (HALT-C Lab Model) HR (95% CI)
Metabolic Tests							
AP Cl		146	35	$\leq 0.28 \text{ mL kg}^{-1} \text{ min}^{-1}$	3.62 (1.83 – 7.14)	3.0 (1.5–5.9)	1.8 (0.8–4.0)
		207	50	$\leq 0.04 \text{ h}^{-1}$	2.67 (1.53 – 4.66)	1.9 (1.1–3.4)	1.2 (0.6–2.2)
GEC		223	53	$\leq 4.32 \text{ mg kg}^{-1} \text{ min}^{-1}$	2.21 (1.29 – 3.78)	1.8 (1.0–3.1)	1.3 (0.7–2.3)
MEGX _{15min}		217	48	$\leq 9.0 \text{ ng mL}^{-1}$	2.50 (1.42 – 4.40)	2.2 (1.2–3.9)	0.9 (0.4–1.8)
MBT		185	40	≤ 48	5.92 (3.01 – 11.66)	4.4 (2.2–8.8)	2.9 (1.4–6.1)
Cholate Clearance and Shunt							
CA Cl _{iv}		224	54	$\leq 3.59 \text{ mL kg}^{-1} \text{ min}^{-1}$	2.87 (1.68 – 4.91)	2.2 (1.3–3.8)	1.2 (0.6–2.3)
CA Cl _{oral}		224	54	$\leq 9.47 \text{ mL kg}^{-1} \text{ min}^{-1}$	6.52 (3.63 – 11.71)	4.0 (2.1–7.9)	2.8 (1.4–5.7)
CA Shunt		225	54	$\geq 46\%$	3.98 (2.28 – 6.92)	2.4 (1.3–4.3)	1.8 (1.0–3.5)
SPECT Liver-Spleen Scan							
PHM		223	53	≤ 94.5	4.97 (2.83 – 8.74)	2.3 (1.2–4.5)	2.2 (1.2–4.1)
Spleen Volume		219	52	$\geq 5.93 \text{ mL kg}^{-1}$	4.16 (2.38 – 7.26)	1.7 (0.9–3.2)	2.2 (1.2–4.0)

HR - Hazard Ratio

LCI - Lower Confidence Interval (95%)

UCI - Upper Confidence Interval (95%)