

The spectrum of hepatic functional impairment in compensated chronic hepatitis C: results from the Hepatitis C Anti-viral Long-term Treatment against Cirrhosis Trial¹

G. T. EVERSON*, M. L. SHIFFMAN†, T. R. MORGAN‡,§, J. C. HOEFS‡,§, R. K. STERLING†, D. A. WAGNER¶, C. C. KULIG*, T. M. CURTO** E. C. WRIGHT†† & THE HALT-C TRIAL GROUP

*Section of Hepatology, Division of Gastroenterology and Hepatology, University of Colorado School of Medicine, Denver, CO, USA; †Division of Gastroenterology, Hepatology, and Nutrition, Hepatology Section, Virginia Commonwealth University Health System, Richmond, VA, USA; ‡Division of Gastroenterology, University of California – Irvine, Irvine, CA, USA; §Gastroenterology Service, VA Long Beach Healthcare System, Long Beach, CA, USA; ¶Metabolic Solutions, Inc., Nashua, NH, USA; **New England Research Institutes, Watertown, MA, USA; ††Office of the Director, Department of Health and Human Services, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD, USA

Correspondence to:
Prof. G. T. Everson, University of Colorado Health Sciences Center, 4200 East 9th Avenue, B-154, Denver, CO 80262, USA.
E-mail: greg.everson@uchsc.edu

¹This is publication number 18 from the HALT-C Trial Group.

Publication data

Submitted 19 December 2007
First decision 22 January 2008
Resubmitted 2 February 2008
Accepted 2 February 2008
Epub Online Accepted 8 February 2008

SUMMARY

Background

The spectrum of functional impairment in patients with compensated chronic hepatitis C is incompletely defined.

Aim

To define hepatic impairment by quantitative tests (quantitative liver function tests) and correlate results with disease severity in patients with chronic hepatitis C.

Methods

We studied 285 adult patients with chronic hepatitis C prior to treatment in the Hepatitis C Anti-viral Long-term Treatment against Cirrhosis Trial; 171 had Ishak fibrosis stages 2–4 (fibrosis) and 114 had stage 5 or 6 (cirrhosis). None had had clinical decompensation. A battery of 12 quantitative liver function test assessed the spectrum of hepatic microsomal, mitochondrial and cytosolic functions, and hepatic and portal blood flow.

Results

Twenty-six to 63% of patients with fibrosis and 45–89% with cirrhosis had hepatic impairment by quantitative liver function test; patients with cirrhosis had the greatest impairment (*P*-value ranging from 0.15 to <0.0001). Cholate Cl_{oral} , cholate shunt and perfused hepatic mass correlated with cirrhosis, stage of fibrosis ($r = -0.51, +0.49, -0.51$), varices and variceal size ($r = -0.39, +0.36, -0.41$). PHM < 95 and cholate shunt >35% identified 91% of patients with medium- or large-sized varices.

Conclusions

Hepatic impairment is common in compensated patients with fibrosis or cirrhosis because of chronic hepatitis C. Cholate shunt, and cholate Cl_{oral} and perfused hepatic mass, identify patients at risk for cirrhosis or varices.

Aliment Pharmacol Ther 27, 798–809

INTRODUCTION

In routine clinical practice, chronic hepatitis C (CHC) is monitored by history and physical examination, standard blood testing, ultrasonography and liver biopsy. Goals of monitoring include definition of disease severity, assessment for progression and evaluation of the impact of treatments or interventions. Patients with late stages of disease are identified by deterioration in laboratory tests and development of clinical complications, but patients with earlier stages of disease have stable laboratory tests and no clinical findings despite hepatic functional impairment and portal hypertension.¹ Prior to laboratory or clinical deterioration, the progression of CHC from fibrosis to cirrhosis disrupts hepatocyte function, distorts the hepatic architecture, and alters hepatic and portal blood flow. Therefore, tests measuring hepatic function, blood flow or mass might better define the spectrum of hepatic impairment in fibrotic stages of CHC prior to obvious laboratory or clinical deterioration.

Hepatic metabolism, blood flow, portal-systemic shunt and perfused mass can be defined by administration of test compounds and measuring clearance or quantifying metabolites.²⁻⁵ These tests are collectively categorized as quantitative liver function tests (QLFTs). Clearances of certain compounds, such as aminopyrine, antipyrine, caffeine, erythromycin and formation of monoethylglycine xylidide (MEGX) from lidocaine, assess hepatic metabolism. In contrast, clearances of others, such as bile acids, lidocaine, propranolol, nitroglycerin and indocyanine green, assess hepatic or portal blood flow. Simultaneous measurement of clearances of orally and intravenously administered cholate assesses portal-systemic shunting (cholate shunt).⁶ Hepatic uptake of intravenously administered 99m-technetium (99Tcm) sulphur colloid, measured by Single Photon Emission liver-spleen scan (SPECT-LSS), defines perfused hepatic mass (PHM).^{7, 8}

The primary goal of our study was to define the severity of hepatic impairment in a cohort of patients with CHC who had fibrosis, including cirrhosis, but who lacked biochemical or clinical decompensation. In this study, we defined hepatic impairment from a battery of QLFTs. This battery assessed hepatic metabolic capacity, hepatic blood flow, portal blood flow, portal-systemic shunt and PHM. Our study subjects were patients with CHC who were participants in the Hepatitis C Anti-viral Long-term Treatment against Cirrho-

sis (HALT-C) Trial.⁹ They had Ishak fibrosis scores from 2 to 6, and, most notably, none had prior clinical decompensation. Finally, because HALT-C patients were comprehensively evaluated, we were able to examine relationships of QLFTs to standard laboratory tests, liver histology, and ultrasonographic and endoscopic findings.

MATERIALS AND METHODS

This study and associated consent forms were approved by the National Institute of Diabetes and Digestive and Kidney Disease, US Food and Drug Administration (FDA), institutional review boards, General Clinical Research Centers and other regulatory bodies within the participating centres. The study was conducted according to the principles of the Declaration of Helsinki regarding proper procedures for human research. All subjects participating in this study had signed individual informed consents for both the main HALT-C trial and this HALT-C ancillary study.

Three HALT-C clinical centres participated in the study (University of Colorado Health Sciences Center, Virginia Commonwealth University and University of California, Irvine). A total of 578 patients were enrolled in the main HALT-C trial at these three centres; and 285 agreed to participate in this ancillary study of QLFTs. The characteristics of the 285 study patients, the 293 patients at the participating centres who did not enrol, and those of the remaining 804 patients from other HALT-C centres are given in Table 1. The only difference was a lower proportion of African-Americans in the studied patients compared with unstudied patients within the three participating centres. Key characteristics of the study population were: mean age 49.9 years, 76% male, mean body mass index (BMI) 29.4, 40% with cirrhosis, 92% hepatitis C virus (HCV) genotype 1 and mean HCV-RNA $6.4 \pm 0.56 \log_{10}$ IU/mL.

Hepatic metabolism and blood flow were measured by clearance techniques using a battery of test compounds. Microsomal function was assessed by caffeine, antipyrine and lidocaine (generation of monoethylglycylxylidide, MEGX), mitochondrial function by methionine, cytosolic function by galactose, and blood flow and relative hepatic perfusion using stable isotopes of cholate and radiolabelled technetium sulphur colloid.

Table 1. Selected baseline patient characteristics and laboratory results

	QLFT sites' study patients (<i>n</i> = 285)	QLFT sites' other patients (<i>n</i> = 293)	Remaining HALT-C patients (<i>n</i> = 804)	<i>P</i> -value (column 1 vs. 2)	<i>P</i> -value (column 1 vs. 3)
Age [years, mean (\pm s.d.)]	49.9 (\pm 7.2)	50 (\pm 7.2)	50 (\pm 7.2)	0.88	0.90
Gender (% male)	76	69	72	0.07	0.16
Race (% African-American)	11	27	14	<0.0001	0.14
Body mass index [kg/m ² , mean (\pm s.d.)]	29.4 (\pm 4.8)	29.5 (\pm 5.7)	29.9 (\pm 5.5)	0.81	0.19
Cirrhosis (%)	40	41	37	0.82	0.40
Bridging fibrosis (%)	60	59	63	0.82	0.40
HCV genotype 1 (%)	92	89	89	0.14	0.13
HCV-RNA level [log ₁₀ of IU/mL, mean (\pm s.d.)]	6.4 (\pm 0.56)	6.4 (\pm 0.56)	6.4 (\pm 0.54)	0.83	0.09
Platelet count [\times 1000/mm ³ , mean (\pm s.d.)]	169 (\pm 69)	175 (\pm 65)	163 (\pm 64)	0.27	0.22

QLFT, quantitative liver function test; s.d., standard deviation; HALT-C, Hepatitis C Anti-viral Long-term Treatment against Cirrhosis; HCV, hepatitis C virus.

Cholates

2,2,4,4-²H cholate (CDN Isotopes Inc., Quebec, Canada, product number D-2452) was administered orally (40 mg) and studied under FDA Investigational New Drug (IND) application 65 123. A solution (20 mg in 5 cm³ NaHCO₃ 1 mmol/ml) of 24-¹³C cholate (CDN Isotopes Inc., product number C-3448) was studied under FDA IND 65 121. The solution of 24-¹³C cholate was passed through micropore filter and transferred to sterile glass vials; sterility and absence of pyrogens were confirmed prior to use.

Galactose

A solution of galactose (Low Endotoxin D-Galactose, Pfanstiehl Laboratories, Inc., Waukegan, IL, USA, product number G-105-1), 30 g in 100 mL sterile water, was administered intravenously and studied under FDA IND 65 107. The solution was passed through micropore filter, transferred to infusion bags and stored under sterile conditions. Sterility and absence of pyrogens were confirmed prior to use.

Caffeine

Caffeine (Ruger Chemical Co., Irvington, NJ, USA, product number 0072-5) was administered orally (300 mg) and studied under FDA IND 65 175.

Antipyrine

Antipyrine (Ruger Chemical Co., product number 0032-5) was administered orally (500 mg) and studied under FDA IND 65 122.

Lidocaine

Pharmaceutical grade lidocaine (Abbott Laboratories, North Chicago, IL, USA), 2% lidocaine HCl was administered intravenously (0.5 mg/kg).

Methionine

1-¹³C-methionine [distributed by Metabolic Solutions, Nashua, NH, USA and manufactured by Isotec, Inc. (division of Sigma Aldrich Company), Miamisburg, OH, USA, product number 49,008-3] was administered orally (200 mg). The amino acid was solubilized in 100 mL water with 200 mg unsweetened Kool Aid (Kraft Foods, Rye Brook, NY, USA) to improve taste and solubility.

Sulphur colloid

[⁹⁹Tcm]-sulphur colloid (*cis*-sulphur colloid, CIS-US, Inc., Bedford, MA, USA) was administered intravenously (5–6 mCi).

Analytical techniques, methods and procedures related to analysis of test compounds from

samples of blood, saliva and breath are given in Appendix A.

Protocol for QLFT testing

Quantitative liver function tests were performed at baseline, prior to any treatment in the HALT-C trial, and no patient was on anti-viral treatment at the time of testing. Participants maintained a caffeine-free diet for 3 days and were studied in the morning after an overnight fast in the General Clinical Research Centers of the participating centres. An indwelling catheter was placed for sampling blood. First, 1-¹³C methionine was administered and breath samples collected every 10 min for 1 h. Subsequently, the sequence of administration of the other test compounds was: lidocaine (2 min), galactose (5 min), oral mixture of 2,2,4,4-²H cholate, caffeine, antipyrine, NaHCO₃ (600 mg) in apple or grape juice (1 min) and 24-¹³C cholate after mixing with 5 mL 25% human serum albumin (Bayer HealthCare Tarrytown, NY, USA, NDC# 0026-0692-16; 1 min). Blood samples for analysis of test compounds or metabolites were obtained at baseline and at 5, 10, 15, 20, 30, 40, 45, 60, 75, 80, 90, 105, 120, 150 and 180 min. Saliva samples were obtained at baseline and at 6, 12, 24, 36, 48 and 60 h. After completion of blood sampling, patients ingested 375 mL Ensure (Abbott Laboratories, Abbott Park, IL, USA) 30 min prior to SPECT-LSS. Serum, plasma and saliva were stored at -20 °C and breath samples were stored at room temperature until analysed.

The number of patients who completed each test were: cholate elimination rate constant (cholate k_{elim}) 282, clearance of orally administered cholate (cholate Cl_{oral}) 282, clearance of intravenously administered cholate (cholate Cl_{iv}) 282 and cholate shunt 282; galactose elimination capacity (GEC) 279; SPECT-LSS 281; MEGX_{30 min} 278; MEGX_{15 min} 274; caffeine elimination rate constant (caffeine k_{elim}) 262; methionine breath test (MBT) 227; antipyrine elimination rate constant (antipyrine k_{elim}) 185 and antipyrine clearance (antipyrine Cl) 182.

Clinical, laboratory, ultrasonographic and histological studies were performed at baseline in all subjects ($N = 285$). Fibrosis was categorized according to Ishak fibrosis score, 2 through 4 (Fibrosis Group, $N = 171$) vs. 5 or 6 (Cirrhosis Group, $N = 114$). To evaluate for varices, upper gastrointestinal endoscopy, was performed approximately 6 months after baseline and only in those who failed to clear HCV-RNA, despite at

least 20 weeks of peginterferon alpha-2a/ribavirin (224 of our 285 patients).⁹ Variceal size was categorized as small, medium or large.

We examined interactions with obesity, insulin resistance and hepatic steatosis as these factors may promote hepatic fibrosis; and with hepatic inflammation, alcohol use and smoking – factors known to influence hepatic function. Obesity was defined by BMI (kg/cm²), insulin resistance by HOMA score,¹⁰ hepatic steatosis by the criteria of Brunt *et al.* (grades 0–4),¹¹ hepatic inflammation by Ishak inflammatory score and alcohol (lifetime intake) and smoking (pack-years) from questionnaires.

Statistical considerations

Distributions of test results were defined by means, medians, standard deviations, ranges, confidence limits and quartiles of results. Associations of QLFTs with cirrhosis and varices were evaluated by logistic regression using the c-statistic (area under receiver operating characteristic curve).^{12, 13} Correlations of QLFTs with Ishak fibrosis score, variceal size and standard laboratory tests were evaluated by regression analysis and Spearman correlation coefficients.^{13, 14}

Quantitative liver function test models were defined by multivariate analysis. Relative strength of models for predicting cirrhosis and varices was determined by first testing for significance and then comparing c-statistics (Statistical Analysis Software, versions 8.2 and 9.1, SAS Institute, Cary, NC, USA). The significance of individual test variables in models was evaluated by stepwise selection using logistic regression analysis.^{12–14}

Correlations of QLFTs with BMI, HOMA score and grade of hepatic steatosis were examined by multivariate regression controlling for cirrhosis. Significant relationships were further analysed after adjustment for the influence of hepatic inflammation, lifetime alcohol use and pack-years of smoking by multiple regression analysis.^{12–14}

RESULTS

Prevalence of abnormalities in standard laboratory tests

Mean (\pm s.d.) for standard laboratory tests overlapped with the normal range: bilirubin 0.78 ± 0.44 mg/dL, INR 1.0 ± 0.10 , albumin 3.8 ± 0.39 g/dl and platelet

count $169\,000 \pm 69\,000/\mu\text{L}$. A minority of patients had abnormal bilirubin, INR or albumin; only 4–16% of the Fibrosis Group and 18–43% of the Cirrhosis Group. Eighteen per cent of the Fibrosis Group and 68% of the Cirrhosis Group had platelet counts $<140\,000/\mu\text{L}$.

Prevalence of abnormal QLFTs

In contrast to standard laboratory tests, a significantly higher percentage of patients had abnormal QLFTs, 26–63% of patients in the Fibrosis Group and 45–89% in the Cirrhosis Group. The spectrum of function, defined by QLFTs, ranged from normal to severely impaired in both Fibrosis and Cirrhosis groups.

In the Fibrosis Group, 58% had cholate shunt $>30\%$, 52% had cholate $\text{Cl}_{\text{oral}} < 1300\text{ mL/min}$ and 38% had $\text{PHM} < 100$. Patients with fibrosis who had abnormal cholate shunt, cholate Cl_{oral} or PHM had significantly higher prevalences of varices and splenomegaly, lower albumin and platelet counts, higher Ishak fibrosis scores and higher Ishak inflammation scores compared with patients with fibrosis with normal cholate shunt, cholate Cl_{oral} or PHM (data not shown).

In the Cirrhosis Group, 89% had cholate shunt $>30\%$, 87% had cholate $\text{Cl}_{\text{oral}} < 1300\text{ mL/min}$ and 80% had $\text{PHM} < 100$. Patients with cirrhosis who had normal cholate shunt, cholate Cl_{oral} or PHM had significantly lower prevalences of varices, portal hypertensive gastropathy and splenomegaly, lower bilirubin and INR, and higher albumin and platelet counts compared with patients with cirrhosis with abnormal cholate shunt, cholate Cl_{oral} or PHM (data not shown).

Relationships of QLFTs to Ishak stage of fibrosis

As anticipated, the hepatic function of the Cirrhosis Group was worse than that of the Fibrosis Group (Figure 1). *P*-values ranged from 0.15 to <0.0001 depending upon the QLFT. Mean and medians of PHM, cholate Cl_{oral} and cholate shunt demonstrated the greatest differences between patients with fibrosis and patients with cirrhosis. The likelihood of cirrhosis increased with worsening quartiles of hepatic function as measured by QLFTs (*P*-values ranged from 0.16 to <0.0001 , Appendix B).

As shown in Table 2, QLFTs, particularly PHM ($r = -0.51$, $P < 0.001$), cholate Cl_{oral} ($r = -0.52$, $P < 0.001$) and cholate shunt ($r = 0.49$, $P < 0.001$), correlated significantly with Ishak fibrosis score. QLFTs

assessing total hepatic blood flow (ranges of $r = -0.16$ to -0.25) and hepatic metabolism (ranges of $r = -0.11$ to -0.31) correlated only weakly with Ishak fibrosis score.

Mean (\pm s.d.) of PHM for Ishak fibrosis stages 2–6 were 104 ± 5.8 ($N = 28$), 102 ± 6.2 ($N = 82$), 99.3 ± 6.9 ($N = 58$), 94.7 ± 9.0 ($N = 52$) and 90.7 ± 9.6 ($N = 61$). The reported lower limit of normal is PHM 100.^{15, 16} At a PHM of 100, the mean Ishak fibrosis score was 3.7 – implying that PHM is abnormal at precirrhotic stages of hepatic fibrosis (Figure 2a).

Mean (\pm s.d.) for cholate shunt for fibrosis stages 2–6 were $27 \pm 9\%$, $33 \pm 13\%$, $37 \pm 15\%$, $44 \pm 15\%$ and $51 \pm 17\%$. The reported upper limit of normal is 30%.¹⁷ At a cholate shunt of 30%, the mean Ishak fibrosis score was 2.6, implying that cholate shunt is abnormal at even earlier stages of hepatic fibrosis than PHM (Figure 2b).

Relationships of QLFTs to varices

Seventy-six of the 224 patients who underwent endoscopy had oesophageal varices (34%). Of the patients with varices, only 20% had abnormal bilirubin ($>1.2\text{ mg/dL}$), 21% abnormal INR (>1.2), 47% abnormal albumin ($<3.5\text{ g/dL}$) and 67% abnormal platelet count ($<140\,000\text{ platelets}/\mu\text{L}$). Interestingly, only 63% of patients with varices had cirrhosis on liver biopsy and only 51% had splenomegaly on ultrasonography. In contrast, 88% had cholate shunt $>30\%$, 87% had cholate $\text{Cl}_{\text{oral}} < 1300\text{ mL/min}$ and 79% had $\text{PHM} < 100$. The prevalence of varices increased with worsening quartiles of hepatic function as measured by QLFTs (*P*-values ranged from 0.18 to <0.0001 , Appendix B).

Quantitative liver function tests, particularly PHM ($r = -0.41$, $P < 0.001$), cholate Cl_{oral} ($r = -0.39$, $P < 0.001$) and cholate shunt ($r = 0.36$, $P < 0.001$), correlated significantly with variceal size (Table 2). QLFTs assessing total hepatic blood flow (ranges from $r = -0.14$ to -0.19) and hepatic metabolism (ranges from $r = -0.09$ to -0.23) correlated poorly with variceal size.

Varices were classified as medium or large in 22 cases. All patients with medium or large varices had elevated cholate shunt, reduced cholate Cl_{oral} and, all but one, reduced PHM. In contrast, 14% of patients with medium to large varices had normal platelet counts, 23% lacked histological cirrhosis and 45% had

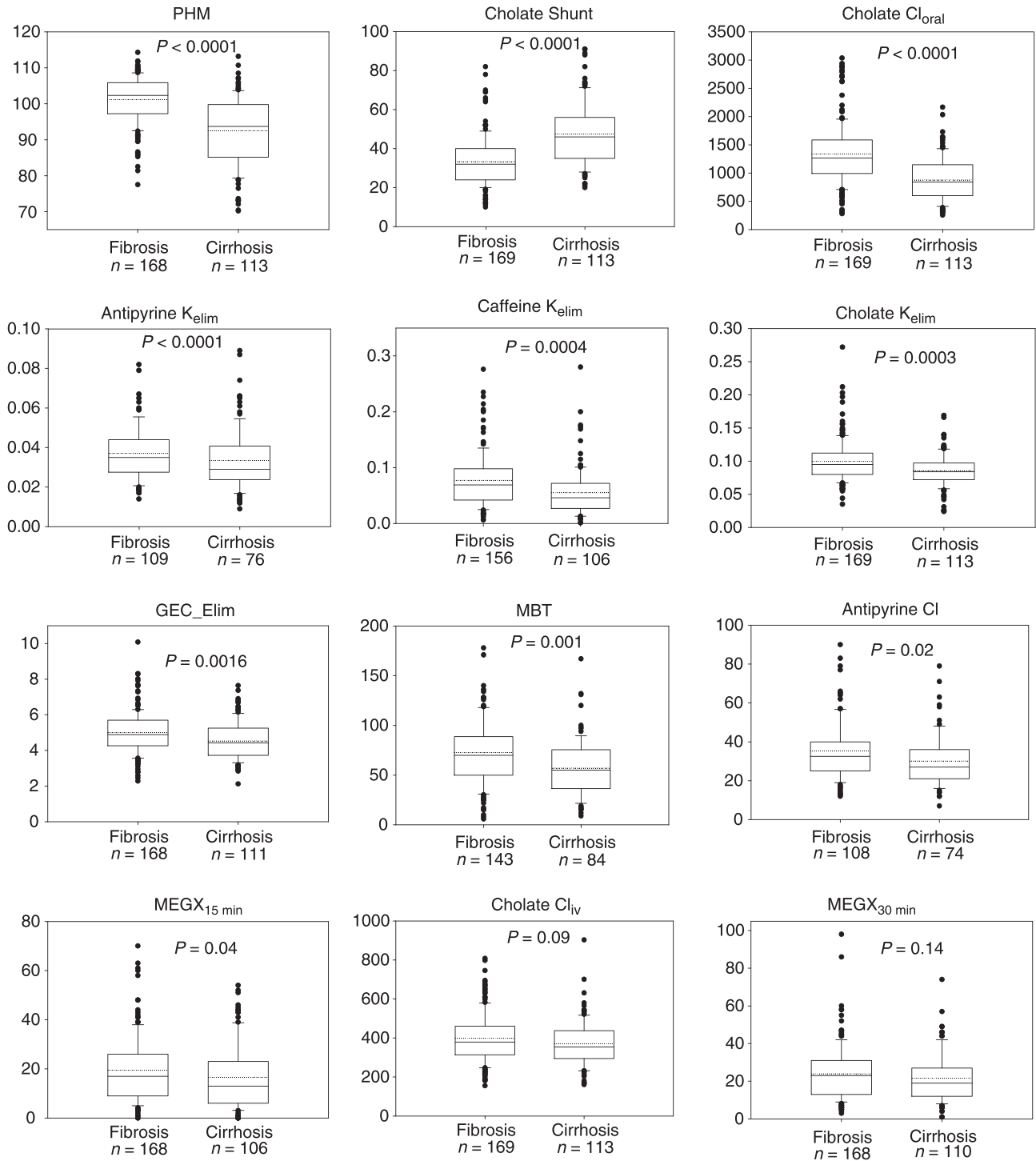


Figure 1. The distributions of results for all quantitative liver function tests (QLFTs) are displayed. Results in patients with Ishak fibrosis scores 2-4 (Fibrosis) are compared to results in patients with Ishak fibrosis scores 5 or 6 (Cirrhosis). Boxes define interquartile ranges (25th to 75th percentiles). Medians are indicated by solid lines and means by dotted lines. Small black bars show 10th and 90th percentiles, and outliers are plotted individually. Nearly all QLFTs, except cholate Cl_{iv} and MEGX_{30 min}, were worse in patients with cirrhosis.

Table 2. Correlations of QLFTs with Ishak fibrosis score and variceal size

QLFT	Fibrosis score (<i>r</i> coefficient)	Variceal size (<i>r</i> coefficient)
PHM		
PHM	-0.51***	-0.41***
Tests of the portal circulation		
Cholate Cl _{oral}	-0.52***	-0.39***
Cholate shunt	+0.49***	+0.36***
Tests of total hepatic blood flow		
Cholate <i>k</i> _{elim}	-0.25***	-0.19**
Cholate Cl _{iv}	-0.16**	-0.14*
GEC	-0.21***	-0.16*
Tests of metabolism		
Microsomal tests		
Antipyrine <i>k</i> _{elim}	-0.31***	-0.20**
Antipyrine Cl	-0.23**	-0.23**
Caffeine <i>k</i> _{elim}	-0.30***	-0.22**
MEGX _{15 min}	-0.13*	-0.15*
MEGX _{30 min}	-0.11	-0.09
Mitochondrial test		
MBT	-0.25***	-0.17*

QLFT, quantitative liver function test; PHG, portal hypertensive gastropathy; PHM, perfused hepatic mass; Cl, clearance; iv, intravenous; MBT, methionine breath test; GEC, galactose elimination capacity; MEGX, monoethylglycine xylidide.

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

normal sized spleens. Patients with no, small, medium or large varices had mean PHMs (\pm s.d.) of 99.7 ± 7.5 ($N = 144$), 94.9 ± 9.0 ($N = 54$), 87.4 ± 8.6 ($N = 18$) and 83.3 ± 7.8 ($N = 4$). Mean (\pm s.d.) cholate shunts were $36 \pm 15\%$ ($N = 147$), $43 \pm 15\%$ ($N = 53$), $52 \pm 13\%$ ($N = 18$) and $73 \pm 14\%$ ($N = 4$) respectively.

Perfused hepatic mass, cholate Cl_{oral} and cholate shunt also correlated significantly with other indicators of advanced liver disease and portal hypertension, including presence and grade of portal hypertensive gastropathy, splenomegaly and standard laboratory tests (data not shown).

QLFT cut-offs for cirrhosis and varices

Our study did not include patients with HCV and minimal stage disease (Ishak Fibrosis score 0 or 1) or healthy volunteers. Despite limiting our study to late stages of fibrosis, QLFTs, particularly PHM, cholate shunt and cholate Cl_{oral}, were able to detect patients with cirrhosis or varices. Examination of the results displayed in Table 3 suggests that cut-offs of PHM 100, cholate shunt 30% and cholate Cl_{oral} 1250 mL/min (the upper limits of the normal ranges for these tests) could be useful in screening for cirrhosis or varices. Cut-offs of PHM 90, cholate shunt

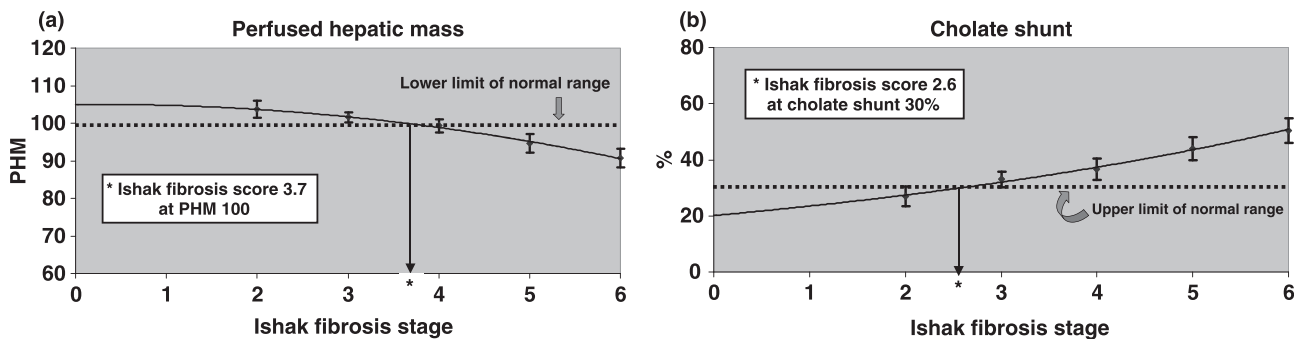


Figure 2. Panel (a) demonstrates the relationship of perfused hepatic mass (PHM) to Ishak fibrosis stage, in reference to the normal range for PHM. As Ishak fibrosis stage increases, PHM decreases. The trendline, fit by nonlinear regression of mean PHM vs. Ishak fibrosis stage, indicates that Ishak fibrosis score is 3.7 at a mean PHM of 100, the lower limit of the normal range. Panel (b) demonstrates the relationship of cholate shunt to Ishak fibrosis score, in reference to the normal range for cholate shunt. As Ishak fibrosis score increases, cholate shunt increases. The trendline, fit by nonlinear regression of mean cholate shunt vs. Ishak fibrosis stage, indicates that Ishak fibrosis score is 2.6 at a mean cholate shunt of 30%, the upper limit of the normal range. Error bars in both panels indicate the 95% confidence limits for the means of Ishak fibrosis score.

Table 3. Sensitivity, specificity and PPV and NPV for cirrhosis and varices based upon cut-offs for PHM, cholate shunt and cholate Cl_{oral}

Test cut-offs	Cirrhosis				Varices			
	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
PHM (ratio)								
110	98	4	41	78	99	5	35	88
105	94	31	48	88	88	24	37	80
100*	80	61	58	82	79	53	47	83
95	55	82	68	73	63	78	59	80
90	34	92	75	68	33	86	56	72
85	26	96	83	66	24	91	58	70
Cholate shunt (%)								
20	99	11	42	95	96	7	35	79
30†	88	43	51	84	87	36	41	84
40	64	73	61	75	64	65	49	78
50	37	91	72	68	37	86	57	73
60	20	95	74	64	17	92	52	68
70	12	98	82	63	12	96	60	68
Cholate Cl_{oral} (mL/min)								
1750	98	17	44	94	97	13	36	90
1500	93	29	46	86	95	23	39	89
1250*	84	51	53	83	84	43	43	84
1000	67	74	63	77	68	66	50	80
750	41	88	69	69	43	84	58	74
500	19	96	76	64	20	93	60	69

PHM, perfused hepatic mass; Cl_{oral} , clearance of the orally administered [2,2,4,4- ^2H] cholate; PPV, positive predictive value; NPV, negative predictive value.

* Lower limit of normal range.

† Upper limit of normal range.

40% and cholate Cl_{oral} 1000 mL/min decrease the sensitivity but increase the specificity for diagnosis of cirrhosis and varices. Cut-offs of PHM < 95 or cholate shunt >35% identified 91% (20 of 22) of the patients with medium- or large-sized varices. A cut off of PHM < 90 identified 55% (12 of 22) and a cut off of cholate shunt >40 identified 86% (19 of 22) of the patients with medium- or large-sized varices.

Analysis of covariates

Selected covariates that might affect the relationships of QLFTs with fibrosis (BMI, HOMA score and grade of hepatic steatosis) or influence the QLFT measurements (hepatic inflammation, alcohol use and smoking) were also examined. By multivariate analysis, the relationships of PHM, cholate Cl_{oral} and cholate shunt with

hepatic fibrosis and cirrhosis were preserved after controlling for all of the above covariates.

However, we did observe significant relationships of BMI with GEC ($P < 0.0001$) and MBT ($P < 0.04$), HOMA score with AP k_{elim} ($P < 0.0001$), AP Cl ($P = 0.06$), MBT ($P = 0.021$) and PHM ($P < 0.04$), and grade of hepatic steatosis with AP Cl ($P = 0.03$) and MBT ($P = 0.0012$), after controlling for cirrhosis and other covariates. The latter findings suggest that the relationships of metabolic QLFTs with fibrosis and cirrhosis may be influenced by additional factors, other than fibrosis, that alter hepatic metabolism.

Models to predict cirrhosis and varices

We next determined whether QLFTs improved prediction of cirrhosis or varices beyond that achievable by the combination of bilirubin, albumin, INR and

platelet count. The combination of standard laboratory tests yielded *c*-statistics of 0.83 and 0.74 for prediction of cirrhosis and varices. Models incorporating QLFTs with standard laboratory tests had the best prediction for cirrhosis and varices. The top models for both cirrhosis (*c*-statistic 0.87, 95% CI: 0.82–0.91) and varices (*c*-statistic 0.79, 95% CI: 0.72–0.86) included PHM, cholate shunt (or cholate Cl_{oral}) and MEGX_{15 min}. By stepwise logistic regression, PHM, cholate Cl_{oral} and cholate shunt were consistently significant variables in all models. Of interest, the addition of histological diagnosis of cirrhosis had no effect in any model in diagnosing varices.

DISCUSSION

This study represents the most comprehensive evaluation of quantitative tests of liver function in the largest cohort of patients prior to clinical decompensation. Our study patients were enrolled in HALT-C and representative of the whole HALT-C cohort.⁹ These patients had significant fibrosis or clinically compensated cirrhosis and had failed prior interferon-based therapy. Even though 40% of our patients had cirrhosis, standard laboratory assessment was generally in the normal range and none had experienced ascites, variceal haemorrhage, encephalopathy or hepatocellular carcinoma. Disease severity was assessed by clinical, laboratory, virological, histological and endoscopic studies. As a result, we were able to examine key relationships of QLFTs with all major components of clinical assessment of patients with CHC. We assessed a broad range of hepatic functions by simultaneously performing 12 QLFTs using eight test compounds. The size of the study population and comprehensive evaluation allowed us to determine the value of quantitative tests of liver function.

Despite the stable, compensated characteristics of our study population, we discovered significant hepatic impairment in a high percentage of patients – a finding that was not appreciated by standard laboratory tests. Not surprisingly, QLFTs were worse in patients with cirrhosis and the degree of hepatic impairment measured by QLFTs correlated with Ishak stage of fibrosis. Our results are novel because they represent the first description of these relationships using a broad array of QLFTs in a group of patients without clinical or biochemical decompensation. Thus, functional assessment by QLFTs at earlier stages of disease may be more sensitive than standard clinical

assessment in identifying those patients with hepatic impairment – patients who may be most at risk for future clinical decompensation.

By comparing 12 QLFTs, we defined the relative value of individual QLFTs in identifying patients with cirrhosis and varices. QLFTs assessing the portal circulation (cholate Cl_{oral} and cholate shunt) or PHM correlated better than QLFTs of hepatic metabolism with cirrhosis, stage of fibrosis, varices and grade of varices. They also correlated with other surrogates for portal hypertension, such as portal hypertensive gastropathy (data not shown), spleen size (data not shown) and platelet count. Given these results, cholate Cl_{oral} , cholate shunt and PHM may be more promising as diagnostic tests than QLFTs of hepatic metabolism in identifying patients with cirrhosis or varices.

This study also defined cut-offs that might have potential clinical utility. PHM ≤ 100 identified the population at risk for cirrhosis and varices; and PHM < 95 identified patients at risk for moderate to large varices. In prior studies, PHM correlated with stage of fibrosis, Child-Turcotte-Pugh score, peritoneoscopy score, explant histology and splenomegaly.^{7, 8, 15, 16} Cholate shunt $> 30\%$ identified the population at risk for cirrhosis and varices; and, cholate shunt > 35 or 40% identified patients with moderate to large varices. In a prior study of patients with compensated cirrhosis, cholate shunt $\geq 40\%$ predicted risk for future decompensation.¹⁷ We conclude that PHM, cholate shunt and cholate Cl_{oral} may be useful for monitoring disease progression and selecting patients for screening for varices by standard or capsule endoscopy.

Our findings call into question the reliance on liver biopsy as the gold standard for assessment of end-stage liver disease. Within our Fibrosis Group, the patients with the greatest impairment in PHM, cholate shunt or cholate Cl_{oral} were more likely to have varices, splenomegaly and abnormalities of standard laboratory tests. Within our Cirrhosis Group, the patients with relative preservation of function defined by PHM, cholate shunt or cholate Cl_{oral} were less likely to have varices, portal hypertensive gastropathy, splenomegaly and abnormalities of standard laboratory tests. We also found that some patients with medium to large varices lacked histological evidence for cirrhosis. These results indicate that sampling error inherent in liver biopsy¹⁸ may lead to inappropriate conclusions regarding stage and severity of liver disease. Supporting this conclusion is one review showing interobserver disagreement in stage of fibrosis on liver biopsies of 10–30%,

intraobserver disagreement of 10–40% and underestimation of cirrhosis of 15–30%.¹

Perfused hepatic mass, cholate Cl_{oral} or cholate shunt may be preferred to liver biopsy in the assessment of severity of liver disease because these tests are not only non-invasive, but also quantify relative perfusion of the liver or the portal circulation. Recent studies show that hepatic venous pressure gradient (HVPG), a measure of the portal circulation, may define disease progression, risk of varices and risk of clinical decompensation.^{19, 20} Ripoll and colleagues measured HVPG in 213 patients with compensated cirrhosis and found that HVPG was independently associated with risk of developing varices or clinical decompensation.¹⁹ Samonakis *et al.*, found that HVPG ≥ 6 mmHg was associated with risk of disease progression and clinical decompensation in recipients of liver transplants.²⁰ PHM, cholate Cl_{oral} and cholate shunt may be preferred over HVPG because they are less invasive, less time-consuming and less costly. In addition, HVPG only assesses portal pressure. In contrast, PHM, cholate clearances and cholate shunt assess total hepatic blood flow, portal blood flow and portal-systemic shunt.

Perfused hepatic mass, cholate Cl_{oral} and cholate shunt correlated with cirrhosis and complications of portal hypertension after controlling for BMI, HOMA score, hepatic steatosis and hepatic inflammation. The clinical implication of these findings is that PHM, cholate Cl_{oral} and cholate shunt might also be useful in conditions other than CHC, such as NASH or alcoholic liver disease.

Although the major thrust of our study was quantification of severity of hepatic impairment according to severity of fibrosis, additional intriguing relationships were uncovered. After controlling for cirrhosis and other covariates, we observed that BMI, HOMA score and grade of hepatic steatosis were associated with impairment of selected hepatic microsomal, mitochondrial and cytosolic functions. Reports from the literature support our findings and suggest that HCV may impair hepatic metabolism, independent of fibrosis. For example, blood concentrations of drugs metabolized by hepatic microsomes (ciclosporin, tacrolimus and HIV protease inhibitors) are increased in patients infected with HCV.^{21–23} In addition, clearance of HCV with anti-viral therapy in transplant recipients enhances hepatic metabolism and reduces plasma levels of both ciclosporin and tacrolimus.²⁴ Cellular and molecular studies indicate that HCV proteins bind and interfere with function of hepatic microsomes.²⁵ Col-

lectively, these observations suggest that HCV may directly interfere with key hepatic metabolic pathways.

In conclusion, in our patients with CHC with advanced fibrosis or compensated cirrhosis, hepatic functional impairment, measured by QLFTs, was common, often undetected by standard laboratory tests, worse in patients with cirrhosis and correlated with stage of fibrosis. QLFTs measuring the portal circulation or hepatic perfusion (cholate Cl_{oral} , cholate shunt and PHM) were superior to QLFTs measuring hepatic metabolism. In regression models, cholate Cl_{oral} , cholate shunt and PHM were additive to standard laboratory tests in identifying patients with cirrhosis or varices; these tests were predictive of not varices alone, but also variceal size. We conclude that QLFTs, particularly cholate shunt, cholate Cl_{oral} and PHM, define disease severity, detect functional impairment not appreciated by routine laboratory tests, and may be useful in the assessment of patients with chronic liver disease.

ACKNOWLEDGEMENTS

Declaration of personal interests: The authors wish to acknowledge the contributions of our co-investigators, study coordinators and staff at each of the participating institutions as follows: University of Colorado School of Medicine, Denver, CO (Contract N01-DK-9-2327, Grant M01RR-00051): Jennifer DeSanto RN, Carol McKinley RN, Brenda Easley RN, Shannon Lauriski BS, Stephanie Shea BA, Michelle Jaramillo and Marcelo Kugelmas MD. M. Kugelmas' current address: South Denver Gastroenterology, Englewood, CO. University of California – Irvine, Irvine, CA (Contract N01-DK-9-2320, Grant M01RR-00827): Muhammad Sheikh MD, Norah Milne MD, Choon Park RN, William Rietkerk, Richard Kesler-West. Virginia Commonwealth University Health System, Richmond, VA (Contract N01-DK-9-2322, Grant M01RR-00065): Charlotte Hofmann RN, Paula Smith RN. New England Research Institutes, Watertown, MA (Contract N01-DK-9-2328): Michael Doherty MS, Kristin K. Snow ScD, N. Salla Ba MD and Marina Mihova MHA. *Declaration of funding interests:* This study was supported by the National Institute of Diabetes & Digestive & Kidney Diseases (contract numbers are listed below). Additional support was provided by the National Institute of Allergy and Infectious Diseases, the National Cancer Institute, the National Center for Minority Health and Health

Disparities and by General Clinical Research Center grants from the National Center for Research Resources, National Institutes of Health (grant numbers are listed below). Additional funding to conduct this study was supplied by Metabolic Solutions, Inc. and by Hoffmann-La Roche, Inc., through a Cooperative Research and Development Agreement with the National Institutes of Health. Financial relationships of the authors with Hoffmann-La Roche, Inc., are as follows: G. T. Everson is a consultant, on the speaker's bureau and receives research support; M. L. Shiffman is a consultant, on the speaker's bureau and receives research support; T. R. Morgan is on the speaker's bureau and receives research support; J. C. Hoefs is on the speaker's bureau and R. K. Sterling is a consultant, on the speaker's bureau and receives research support; other financial relationships related to this project are: G. T. Everson, M. L. Shiffman and T.R. Morgan receive research support from Metabolic Solutions, Inc.; R. K. Sterling is a consultant and receives support from Wako Diagnostics and D. A. Wagner has equity and intellectual property rights in Metabolic Solutions, Inc. G. T. Everson and UCHSC have filed US Patent Application No. 60/647,689, 'Methods for Diagnosis and Intervention of Hepatic Disorders', 26 January 2005, and

International Application Number PCT/US2006/003132 as published under the Patent Cooperation Treaty, World Intellectual Property Organization, International Patent Classification A61K 49/00 (2006.01), International Publication Number WO 2006/081521 A2, 3 August 2006. Authors with no financial relationships to disclose are C. Kulig, T. M. Curto and E. C. Wright.

SUPPLEMENTARY MATERIAL

The following supplementary material is available for this article:

Appendix A. Analytical methods and calculations

Appendix B. Quartiles of QLFTs with numbers of patients (*N*) and percentages (%) for cirrhosis and varices

This material is available as part of the online article from:

<http://www.blackwell-synergy.com/doi/abs/10.1111/j.1365-2036.2008.03639.x>

Please note: Blackwell Publishing are not responsible for the content or functionality of any supplementary materials supplied by the authors. Any queries (other than missing material) should be directed to the corresponding author for the article.

REFERENCES

- Fontana RJ, Lok ASF. Noninvasive monitoring of patients with chronic hepatitis C. *Hepatology* 2002; **36**: 557–64.
- Lotterer E, Hogel J, Gaus W, Fleig W, Bircher J. Quantitative liver function tests as surrogate markers for endpoints in controlled clinical trials: a retrospective feasibility study. *Hepatology* 1997; **26**: 6.
- Reichen J. Assessment of hepatic function with xenobiotics. *Semin Liver Dis* 1995; **15**: 3.
- Tanaka E, Breimer DD. *In vivo* function tests of hepatic drug-oxidizing capacity in patients with liver disease. *J Clin Pharm Ther* 1997; **22**: 237–49.
- Figg W, Dukes G, Lesesne H, *et al.* Comparison of quantitative methods to assess hepatic function: Pugh's classification, indocyanine green, antipyrine, and dextromethorphan. *Pharmacotherapy* 1995; **15**: 6.
- Everson GT, Martucci MA, Shiffman ML, *et al.* Portal-systemic shunting in patients with fibrosis or cirrhosis due to chronic hepatitis C: the minimal model for measuring cholate clearances and shunt. *Aliment Pharmacol Ther* 2007; **26**: 401–10.
- Hoefs JC, Wang F, Kanel G, Braunstein P. The liver-spleen scan as a quantitative liver function test: correlation with liver severity at peritoneoscopy. *Hepatology* 1995; **22**: 1113–21.
- Hoefs JC, Wang F, Kanel G. Functional measurement of the non-fibrotic hepatic mass in cirrhotic patient. *Am J Gastroenterol* 1997; **92**: 2054–8.
- Lee WM, Dienstag JL, Lindsay KL, *et al.* Evolution of the HALT-C Trial: pegylated interferon as maintenance therapy for chronic hepatitis C in previous interferon nonresponders. *Control Clin Trials* 2004; **25**: 472–92.
- Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner AC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* 1985; **28**: 412–9.
- Brunt EM, Janney CG, Di Bisceglie AM, Neuschwander-Tetri BA, Bacon BR. Nonalcoholic steatohepatitis: a proposal for grading and staging the histological lesions. *Am J Gastroenterol* 1999; **94**: 2467–74.
- Hosmer DW, Lemeshow S. *Applied Logistic Regression*, 2nd edn. New York, NY: John Wiley and Sons, 2000.
- SAS Institute, Inc., *SAS/STAT® 9.1 User's Guide*, Cary, NC: SAS Institute, Inc., 2004.
- Rosner B. *Fundamentals of Biostatistics*, 3rd edn. Belmont, CA: Duxbury Press, 1990.
- Hoefs J, Chang K, Wang F, Kanel G, Morgan T, Braunstein P. The perfused Kupffer cell mass: correlation with

- histology and severity of CLD. *Dig Dis Sci* 1995; 40: 552–60.
- 16 Hoefs JC, Wang F, Lilien D, Walker B, Kanel B. A novel, simple method of functional spleen volume calculation by liver-spleen scan. *J Nucl Med* 1999; 40: 1745–55.
 - 17 Shrestha R, McKinley C, Showalter R, *et al.* Quantitative Liver Function Tests (QLFTs) define the functional severity of liver disease in early stage cirrhosis. *Liver Transpl Surg* 1997; 3: 166–73.
 - 18 Bedossa P, Dargere D, Paradis V. Sampling variability of liver fibrosis in chronic hepatitis C. *Hepatology* 2003; 38: 1449–57.
 - 19 Ripoll C, Groszmann R, Garcia-Tsao G, *et al.* Hepatic venous pressure gradient predicts clinical decompensation in patients with compensated cirrhosis. *Gastroenterology* 2007; 133: 481–8.
 - 20 Samonakis DN, Cholonngitas E, Thalheimer U, *et al.* Hepatic venous pressure gradient to assess fibrosis and its progression after liver transplantation for HCV cirrhosis. *Liver Transpl* 2007; 13: 1305–11.
 - 21 Trotter JF, Osborne JC, Heller M, Christian U. Effect of hepatitis C infection on tacrolimus dose and blood levels in liver transplant recipients. *Aliment Pharmacol Ther* 2005; 22: 37–44.
 - 22 Wolffenbittel L, Poli DD, Manfro RC, Goncalves LF. Cyclosporine pharmacokinetics in HCV+ patients. *Clin Transplant* 2004; 18: 654–60.
 - 23 Robertson SM, Scarsi KK, Postelnick MJ, Lynch P. Elevated plasma concentrations of protease inhibitors and nonnucleoside reverse transcriptase inhibitors in patients coinfecting with human immunodeficiency virus and hepatitis B or C: case series and literature review. *Pharmacotherapy* 2005; 25: 1068–72.
 - 24 Kugelmas M, Osgood M, Trotter JF, *et al.* Hepatitis C virus therapy, hepatocyte drug metabolism, and risk for acute cellular rejections. *Liver Transpl* 2003; 9: 1159–65.
 - 25 Perlemuter G, Sabile A, Letteron P, *et al.* Hepatitis C virus core protein inhibits microsomal triglyceride transfer protein activity and very low density lipoprotein secretion: a model of viral-related steatosis. *FASEB J* 2002; 16: 185–94.