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Portal-systemic shunting in patients with fibrosis or cirrhosis due to chronic hepatitis C: the minimal model for measuring cholate clearances and shunt

G. T. EVERSON*, M. A. MARTUCCI*, M. L. SHIFFMAN†, R. K. STERLING†, T. R. MORGAN‡,§, J. C. HOEFS‡,§ & THE HALT-C TRIAL GROUP

*Section of Hepatology, Division of Gastroenterology and Hepatology, University of Colorado School of Medicine, Denver, CO, USA; †Section of Hepatology, Virginia Commonwealth University Medical Center, Richmond, VA, USA; ‡Division of Gastroenterology, University of California – Irvine, Irvine, CA, USA; §Gastroenterology Service, VA Long Beach Healthcare System, Long Beach, CA, USA

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

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SUMMARY

Background
Measurement of portal inflow and portal-systemic shunt using cholate clearances could be useful in monitoring patients with liver disease.

Aim
To examine relationships of cholate clearances and shunt to cirrhosis and varices and to define minimal sampling requirements.

Methods
Five hundred forty-eight studies were performed in 282 patients enrolled in the Hepatitis C Antiviral Long-term Treatment to prevent Cirrhosis (HALT-C) trial. Stable, non-radioactive isotopes of cholate were administered intravenously and orally, clearances (Cl_{iv} and Cl_{oral}) were calculated from [dose/area under curve (*AUC*)] and cholate shunt from [(AUC_{oral}:AUC_{iv}) x ($Dose_{iv}$:$Dose_{oral}$) x 100%].

Results
Cholate Cl_{oral} and cholate shunt correlated with prevalences of both cirrhosis and varices ($P < 0.0001$ for all). Peripheral venous sampling at 5, 20, 45, 60 and 90 min defined the minimal model. Linear regression of cholate shunt determined from five points within 90 min vs. the standard method of 14 points over 3 h yielded slope of 1.0 and intercept 0.5% ($r^2 = 0.98$, $P < 0.0001$). Results were identical in the 189 validation studies (slope 1.0, intercept 0.5%, $r^2 = 0.99$, $P < 0.0001$).

Conclusions
Cholate Cl_{oral} and cholate shunt may be useful in monitoring patients with liver disease. The 5-point model enhances application of cholate Cl_{oral} and cholate shunt in the non-invasive assessment of the portal circulation.

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INTRODUCTION

Chronic liver disease affects over 10 million Americans, and is the 12th leading cause of death in the United States.[1] Chronic hepatitis C (CHC) alone currently affects 4 million Americans and is responsible for 10 000 deaths annually.[2, 3] By 2020, the number of patients with HCV-related cirrhosis will double, incidence of hepatocellular carcinoma will increase by 81%, and HCV-related death will increase by 180%.[2] Although chronic liver disease is a major cause of morbidity and mortality, methods for tracking disease progression are woefully inadequate.

Major clinical complications of chronic liver disease, such as variceal haemorrhage, ascites and encephalopathy, are related to portal hypertension and portal-systemic shunting. Despite this central role, the portal circulation is incompletely defined using standard tests.[4] Platelet count is the one standard test that may correlate with portal hypertension as platelets may be sequestered in the spleen as portal pressure increases. However, platelet count relates only indirectly to portal hypertension and many other factors, such as diminished thrombopoeitin production, intercurrent disease, medications and impaired bone marrow function, may cause thrombocytopenia in patients with chronic liver disease. Furthermore, many patients with advanced fibrosis or cirrhosis, who have portal hypertension, impaired portal blood flow and portal-systemic shunt, lack thrombocytopenia. Other tests of the portal circulation, such as ultrasonographic measurement of spleen size, are insensitive, and still others, such as transjugular measurement of portal pressure, are invasive.

Cholate shunt directly measures first pass hepatic extraction of cholate, a function influenced primarily by portal blood flow and portal-systemic shunt. It is defined as the percentage of orally administered cholate that escapes hepatic extraction from the portal circulation. In recent studies primarily of patients with CHC, cholate shunt correlated with clinical manifestations of portal hypertension and response to anti-viral therapy,[5–8] and was more sensitive than standard laboratory tests in detecting fibrotic stages of liver disease.[5] However, the method for measuring cholate shunt required a sampling period of 3 h and 14 samples of blood.

The primary objectives of the current study were to examine relationships of cholate shunt to cirrhosis and varices and to define the minimal sampling requirements for accurate measurement of cholate shunt in man.

PATIENTS AND METHODS

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

Patients

For this analysis we used 548 studies of 282 patients with CHC enrolled in the Hepatitis C Antiviral Long-term Treatment against Cirrhosis (HALT-C) Trial[9] who participated in the quantitative liver function test (QLFT) ancillary study.[5] HALT-C patients are characterized by hepatic fibrosis (Ishak fibrosis scores 2–4) or compensated cirrhosis (Ishak fibrosis score 5 or 6), non-response to prior courses of anti-viral therapy, and exhibit a broad range of functional impairment[5] and clinical manifestations.[10] These results were compared with results from 32 healthy controls. The first 359 studies of HALT-C patients were used for definition of the minimal model, and the model was validated in the next 189 HALT-C studies.

Test compounds

2,2,4,4-^{2}H cholate (CDN Isotopes Inc., Quebec, Canada, product # D-2452) was administered orally (40 mg) and studied under FDA Investigational New Drug (IND) application no. 65,123. A solution (20 mg in 5 cm^3 $NaHCO_3$ 1 mmol/mL) of 24-^{13}C cholate (CDN Isotopes Inc., product # C-3448) was studied under FDA IND 65,121. The solution of 24-^{13}C cholate was passaged through micropore filter and transferred to sterile glass vials; sterility and absence of pyrogens were confirmed prior to use.

Patient protocol

24-^{13}C cholic acid solution, 20 mg, was first mixed with 5 mL human serum albumin (25% w/v), and then

administered intravenously through an indwelling intravenous catheter over 2 min. 2,2,4,4-^{2}H cholic acid, 40 mg, was dissolved in water, mixed in juice and taken orally at the same time as the intravenous injection. Peripheral venous blood samples for measurement of serum concentrations, were drawn through the indwelling catheter and obtained prior to and 5, 10, 15, 20, 30, 45, 60, 75, 90, 105, 120, 150 and 180 min after administration of cholate isotopes.

Sample preparation

Aliquots of serum (0.5 mL) were dispensed, 1.5 μg of unlabelled cholate was added as internal standard, and cholates were isolated from serum using C18 liquid chromatographic cartridges. The eluate was acidified with concentrated HCl and cholates extracted from the eluate with diethyl ether, methylated, and derivatized to trimethyl-silyl ethers. Isotopes were quantified by isotope dilution – mass spectrometry and selected ion monitoring (*m/z* 458, 459, 462; Agilent GC 6890 MS 5973N, Agilent Technologies, Paulo Alto, CA, USA), equipped with an HP-1 MS 30 m x 25 mm column (Agilent Technologies).[11, 12]

Calculations

A full description of the methods and mathematical models used in curve fitting, measurement of *AUC* and analysis of models is provided in Supplementary Material – Appendix S1.[13–15]

Clearances and shunt fraction

Areas under serum concentration curves (*AUC*s) were measured and clearances and shunt were calculated. Intravenous clearance (Cl_{iv}) was defined by $dose_{24\text{-}13C\ cholate}/AUC_{24\text{-}13C\ cholate}$, oral clearance ($Cl_{oral}$) by $dose_{2,2,4,4\text{-}2H\ cholate}/AUC_{2,2,4,4\text{-}2H\ cholate}$ and cholate shunt by $[AUC_{oral}{:}AUC_{iv}]$ x $[Dose_{iv}{:}Dose_{oral}]$ x 100%.

Statistical analyses

Distributions of test results were defined by mean, median, standard deviation and quartiles spanning the full range of results. Models using reduced sampling were evaluated for accuracy in determination of cholate shunt, by comparison with results from the full 14 samples obtained over 3 h. Correlations of results of models with the full 14-point sampling period were evaluated by linear regression analysis and Spearman correlation coefficients.[16–18]

RESULTS

Serum concentrations of administered isotopes

Mean serum concentrations (±s.d.) after oral and intravenous administration of cholate isotopes in the first 359 studies are illustrated in Figure 1. The mean values and standard deviations of serum concentrations of cholate isotopes reflect the variability of liver disease within this cohort with CHC and fibrosis or compensated cirrhosis. Healthy controls have lower mean values and standard deviations (data not shown). Despite the interindividual variation in serum concentrations, due to varying severity of underlying liver disease, the overall shape of both intravenous and oral clearance curves was generally similar from patient to patient and in healthy controls.

Baseline cholate clearances and shunt

The full range and boundaries for quartiles of results of cholate clearances and shunt in the 282 patients at baseline, prior to entry into the Trial, are shown in Table 1. Clearances progressively declined and shunt progressively increased as results ranged from 'best' to 'worst'. Cholate shunts spanned the entire range of expected result, from the low end of the normal range, 10% ('Best'), to nearly complete shunting, 91% ('Worst').

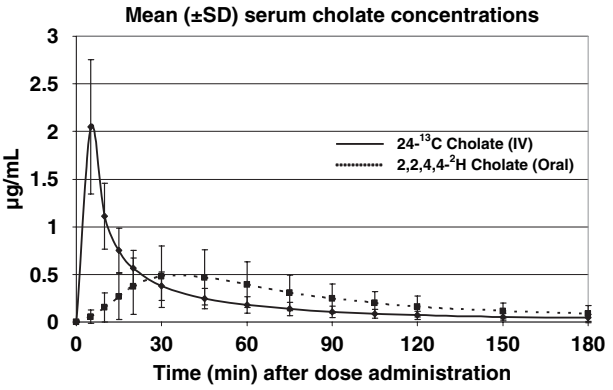

Figure 1. Mean serum concentrations (±s.d.) from peripheral venous blood after intravenous (24-^{13}C cholate) and oral (2,2,4,4-^{2}H cholate) administration of stable isotopes are shown. Intravenous clearance obeyed a tri-exponential function and oral clearance was characterized by absorptive and elimination phases.

Table 1. Range of results for cholate clearances and cholate shunt in study patients

	Boundaries for quartiles of test results*				
	Best	25th	50th	75th	Worst
Cholate Cl_{iv} (mL/min)	903	457	367	305	155
Cholate Cl_{oral} (mL/min)	3036	1427	1087	768	255
Cholate shunt (%)	10	27	36	48	91

Cl_{iv}, clearance after intravenous administration of cholate; Cl_{oral}, clearance after oral administration of cholate.
* The ranges of results of baseline tests in the 282 study patients are defined by boundaries for the quartiles of results. The column headed 'Best' lists the maximum values for cholate clearances and minimum value for cholate shunt; the column headed 'Worst' lists the minimum values for cholate clearances and maximum value for cholate shunt. Clearances decline and shunt increases from 'Best' to 'Worst' quartiles. Cl_{iv} is the clearance after intravenous administration of cholate and Cl_{oral} is the clearance after oral administration of cholate.

Results in 32 healthy controls were (mean ± s.d., range): Cl_{iv} 390 ± 136, 155–873 mL/min; Cl_{oral} 2173 ± 677, 1369–3856 mL/min; and shunt 18.5 ± 5.5, 8.0–28.5%. The range of Cl_{iv} of HALT-C patients (Table 1) completely overlapped with Cl_{iv} for these healthy controls. In contrast, approximately 70% of HALT-C patients exceeded the normal limits for Cl_{oral} of 1300 mL/min and shunt of 30% (Table 1). Consequently, both Cl_{oral} and shunt, but not Cl_{iv}, may be useful for defining risk of cirrhosis and varices.

Correlations of cholate clearances and shunt with cirrhosis and varices

One hundred and thirteen of the 282 study patients had cirrhosis. The numbers of patients and percentages of patients with cirrhosis within quartiles of test results are given in Table 2, panel A. Cl_{oral} was ≤1300 mL/min and shunt was ≥30% in 87% and 88% of patients with cirrhosis. The prevalence of cirrhosis increased as Cl_{oral} decreased ($P < 0.0001$) and shunt increased ($P < 0.0001$).

Seventy-five of 222 study patients that underwent endoscopy had varices. Sixty-five of these 75 patients (87%) had Cl_{oral} ≤1300 mL/min and 66 (88%) had shunt ≥30%. In contrast, only 48 of the 75 patients with varices (64%) had biopsy-proven cirrhosis. The varices of patients with normal Cl_{oral} ($n = 10$) and shunt ($n = 9$) were all classified as small. The latter patients tended to have higher platelet counts, normal spleen size, and only one of the 10 with normal Cl_{oral} and two of the nine with normal shunt had cirrhosis on biopsy.

Prevalence of varices increased as Cl_{oral} decreased ($P < 0.0001$) and shunt increased ($P < 0.0001$; Table 2, panel B). Twenty-two of the 75 patients with varices had medium ($n = 15$) or large ($n = 7$) varices. All had Cl_{oral} ≤1300 mL/min and shunt ≥30% and all of the seven patients who had large varices had shunt ≥45%. In contrast, only 17 of the 22 patients with medium to large varices (77%) had biopsy-proven cirrhosis. Altogether, these results suggest that Cl_{oral} and shunt may be more sensitive than stage of fibrosis on liver biopsy in detecting patients with varices.

Cl_{oral} and shunt also correlated with other measures of severity of liver disease, including Ishak fibrosis score, variceal size, splenomegaly, portal hypertensive gastropathy and standard laboratory tests, such as bilirubin, INR, albumin and platelet count (data not shown).

Characteristics of clearance of intravenously administered 24-^{13}C cholate

The mean serum concentrations of intravenously administered 24-^{13}C cholate for the 14 time points are illustrated in Figure 2. Three phases of clearance were identified: 0–20, 20–45 and 45–180 min. Equations were defined for each of the three phases of elimination, bracketed by the four time points of 5, 20, 45 and 90 min (see Supplementary Material – Appendix S1 for derivation of equations). The equation defining elimination rate between 45 and 90 min was extrapolated to 180 min. The area under the curve (*AUC*) of plasma concentration vs. time was determined from the four time points by integration of the resultant triexponential equation. The *AUC* of the 4-point model was within 98 ± 1.7% of the *AUC* calculated from all 14 points. In the final combined 5-point model defined below, only the four time points noted above are used for the intravenous curve.

Characteristics of clearance of orally administered 2,2,4,4-^{2}H cholate

The mean serum concentrations of orally administered 2,2,4,4-^{2}H cholate for the 14 time points are illustrated

Table 2. Prevalence of cirrhosis and varices within quartiles of cholate clearances and shunt

	Number of patients within each quartile of test result				Percentage of patients with cirrhosis within each quartile of test result				CMH statistic
	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	*P*-value
Panel A: Prevalence of cirrhosis*									
Cholate Cl_{iv}	71	71	71	69	35%	35%	45%	45%	0.14
Cholate Cl_{oral}	71	70	71	70	17%	27%	49%	67%	<0.0001
Cholate shunt	67	72	68	75	10%	31%	49%	68%	<0.0001
					Percentage of patients with varices within each quartile of test result				
Panel B: Prevalence of varices†									
Cholate Cl_{iv}	55	49	62	56	22%	33%	40%	39%	0.03
Cholate Cl_{oral}	49	52	61	60	14%	25%	34%	57%	<0.0001
Cholate shunt	45	57	55	65	11%	23%	38%	55%	<0.0001

Cl_{iv}, clearance after intravenous administration of cholate; Cl_{oral}, clearance after oral administration of cholate; Q1, the quartile of patients with least impaired function; Q2 and Q3, the quartiles of patients with intermediate functional impairment; Q4, the quartile of patients with most impaired function; CMH, Cochran-Mantel-Haenszel test of significance of the trend in percentage of patients with cirrhosis from Q1 to Q4.

* Two hundred and eighty-two patients underwent testing and 113 of these had cirrhosis. Panel A displays the numbers of patients and prevalences of cirrhosis within quartiles of test results. Prevalences of cirrhosis were lowest in Q1, the quartiles with best test results, and highest in Q4, the quartiles with worst test results. Cholate Cl_{oral} and cholate shunt, but not cholate Cl_{iv}, correlated significantly with prevalence of cirrhosis, which increased progressively from Q1 to Q4; † Two hundred and twenty-two patients underwent endoscopy and 75 had varices. Panel B shows the numbers of patients and prevalences of varices within quartiles of results. Prevalences of varices were lowest in Q1 and highest in Q4. Cholate Cl_{oral} and cholate shunt correlated best with prevalence of varices, which increased progressively from Q1 to Q4.

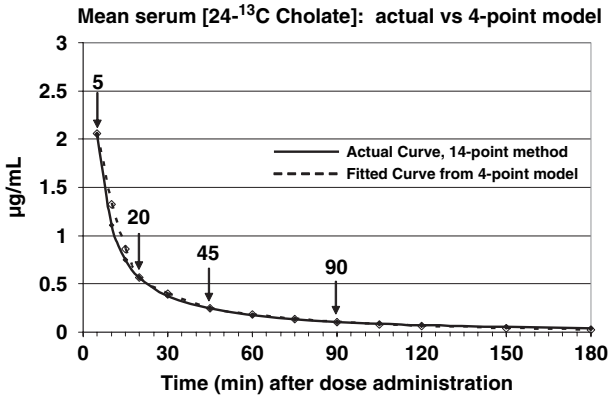

Figure 2. The serum concentrations for intravenously (24-^{13}C cholate) administered cholate are shown. The arrows indicate the four time points for sampling at 5, 20, 45 and 90 min that bracket the three exponential phases of clearance.

in Figure 3. The initial increase in systemic concentration of 2,2,4,4-^{2}H cholate occurred at 5 min and peak concentrations occurred between 30 and 60 min. We analysed various combinations of time points for generation of the oral curve and concluded that sampling at 5, 20, 45, 60 and 90 min would be required to bracket all inflection points in clearance curves from every patient. Cubic spline functions were defined for all time intervals between 0 and 90 min of the oral curve (Supplementary Material – Appendix S1). The final interval from 90 to 180 min was defined by a single exponential function using the mean rate constant of elimination (k_{elim}) for this interval from all 359 studies, 0.01295 min^{-1}.

The *AUC* of plasma concentration vs. time was integrated using the respective spline and exponential functions. The *AUC* using the five time points was within 98 ± 1% of the *AUC* calculated from the 14 time points.

Primary end point: cholate shunt

Cholate shunts calculated from 5 and 14 time points for each individual were nearly identical – failing to

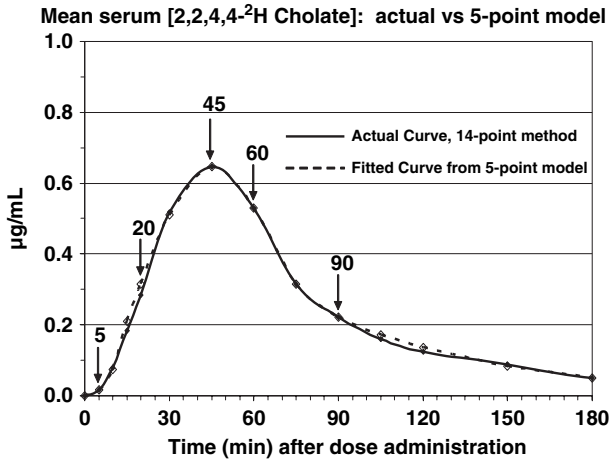

Figure 3. The serum concentrations for orally (2,2,4,4-^{2}H cholate) administered cholate are shown. The arrows indicate the five time points for sampling at 5, 20, 45, 60 and 90 min that define the optimal model for generation of the oral clearance curve.

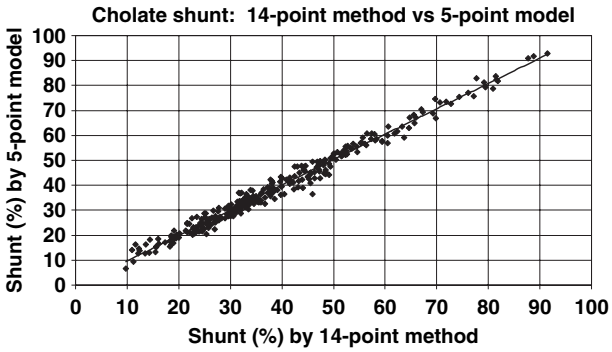

Figure 4. Results of 359 studies of 282 patients with chronic hepatitis C (model building set). Cholate shunt from analyses using the five time points defining the optimal model (*y* axis) are plotted against cholate shunt derived from the 14 point standard method (*x* axis). The slope from linear regression (solid line) was equivalent to the line of identity.

match in only one study due to improper administration of the intravenous dose. Cholate shunt, calculated using five points, was 98 ± 1% of cholate shunt measured with 14 time points. Linear regression of cholate shunts measured by five points vs. 14 points yielded a straight line, indistinguishable from the line of identity (slope 1.0, intercept 0.5%, $r = 0.99$, $P < 0.0001$; Figure 4). Linear regression analysis of results from five and 14 point methods are given in Table 3. Regression analysis of five point vs. 14 point models for shunt and clearances of intravenously or orally administered cholates yielded slopes indistinguishable from the lines of identity and intercepts equivalent to zero.

Evaluation of other models

After defining time period and sampling intervals, we then examined variations in the number of samples required to define cholate shunt by analysing models using 2, 3, 4, 6 and 7 time points. For 2-point models, we used the average k_1 and k_2 values for the exponential elimination rates for the intravenous curve. *AUC*s were calculated by integrating from the intersection of the two elimination rates to respective end points. For the 3-point model, the intravenous curve used one average k elimination rate and calculated the other k elimination rate depending on the points selected. The 4-, 6- and 7-point intravenous curve was generated as described above for the 5-point analysis. All models used the spline functions for the oral curve. The 2-, 3- and 4-point models used 5 min as one time point and all combinations of time points at 20, 45, 60 and 90 min. The 6- and 7-point models used time points at 5, 20, 45, 60 and 90 time points, but also different combinations of time points at 15, 75, 105, 120 and 180 min. The 5-point model proved to be optimal in the determination of cholate shunt (Figure 5).

Validation of 5-point model

To validate the 5-point model, we applied the model prospectively to the next 189 studies. Linear regression analysis of results from five and 14 point methods using this validation set are given in Table 3. Regression analysis of five point vs. 14 point models for shunt and clearances of intravenously or orally administered cholates yielded slopes indistinguishable from the line of identical and intercepts equivalent to zero. Shunt and clearances of intravenously or orally administered cholates were, once again, accurately defined using the 5-point model.

DISCUSSION

A major deficiency of the current clinical assessment of chronic liver disease is the inability of standard laboratory tests to adequately and non-invasively assess hepatic function, the portal circulation and portal-systemic shunt.[4] Quantification of hepatic physiology and pathophysiology by use of test compounds,

Table 3. Comparison of clearances and shunt determined from the 5-point minimal model with results from the standard 14-point method

	Slope	Intercept	*r*	*P*-value
Panel A: Model-building set: results of linear regression analysis*				
Cl_{iv} (mL/min)	0.96	11.7	0.98	<0.0001
Cl_{oral} (mL/min)	0.98	14.8	0.98	<0.0001
Shunt (%)	1.0	0.53	0.99	<0.0001
Panel B: Validation set: results of linear regression analysis†				
Cl_{iv} (mL/min)	0.98	7.7	0.98	<0.0001
Cl_{oral} (mL/min)	1.0	7.9	0.99	<0.0001
Shunt (%)	1.0	0.67	0.99	<0.0001

Cl_{iv}, clearance after intravenous administration of cholate; Cl_{oral}, clearance after oral administration of cholate; *r*, correlation coefficient; *P*, *P*-value of probability.

* The model-building set consisted of the first 359 studies of cholate clearances and shunt; 282 were performed at baseline and an additional 77 in follow-up studies of the same patients. All correlations of results from the 5-point model with results from the 14-point standard method were highly significant. The trendline of linear regression of Cl_{iv}, Cl_{oral} and shunt determined from the 5-point model vs. the standard 14-point method approximated the line of identity; † The validation set consisted of an additional 189 follow-up studies of cholate clearances and shunt performed in the same patients. All correlations of results from the 5-point model with results from the 14-point standard method were highly significant. Once again, the trendline of linear regression of Cl_{iv}, Cl_{oral} and shunt from the 5-point model vs. the standard 14-point method approximated the line of identity. The 5-point model of sampling for only 90 min accurately measures Cl_{iv}, Cl_{oral} and shunt.

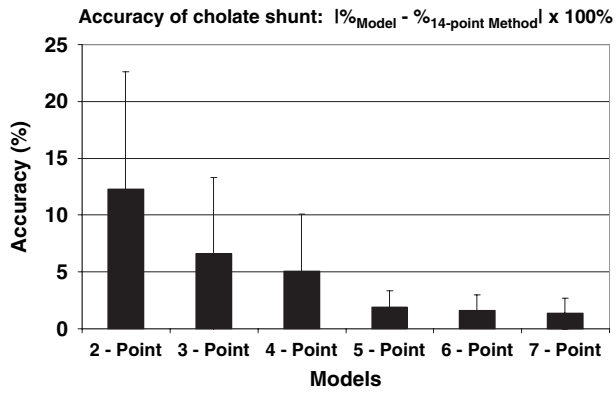

Figure 5. Mean differences (±s.d.) of measurements of cholate shunt with various models using reduced numbers of time points in comparison to the standard 14 point method are shown. Models using <5 points were associated with significant mean error and higher variation. Models incorporating five points or more were equivalent and within 1–2% of the measurement of cholate shunt by the 14 point standard method.

such as cholate, may allow better definition of the portal circulation and enhance the evaluation of patients with chronic liver disease.[19–22]

In this study, we demonstrated that cholate Cl_{oral}, using a cut-off of 1300 mL/min, and shunt, using a cut-off of 30%, may be useful in detecting patients with cirrhosis and varices – both tests were more sensitive than liver biopsy in detection of patients with varices. Previously we reported that cholate shunt and cholate Cl_{oral} correlated with Ishak fibrosis score, variceal size and grade of portal hypertensive gastropathy at endoscopy, spleen size on ultrasonography and routine laboratory tests of liver dysfunction.[5] In addition, other analyses have indicated that cholate shunt and cholate Cl_{oral} may be useful in prediction of response to anti-viral therapy,[6, 7] and risk of future decompensation.[8] They may also define improvements in hepatic function that are undetected by routine laboratory tests.[7] These findings suggest that cholate Cl_{oral} and shunt could have potential clinical utility.

Some patients with small varices had normal Cl_{oral} and shunt. These patients exhibited few signs of portal hypertension – platelet counts and spleen size were generally normal and most had non-cirrhotic liver biopsies. This suggests that in the early stages of portal hypertension, hepatic extraction of cholate may remain normal via compensatory mechanisms that improve portal and hepatic blood flow or enhance the hepatocellular uptake of cholate.

Standard methods for measuring cholate Cl_{oral} and shunt, requiring 14 samples of blood collected over 3 h, are clinically impractical. For these reasons we used mathematical methods to define the minimal sampling

requirements – five time points within 90 min of administration of cholates. Our 5-point model greatly simplifies performance of the test increasing its potential for broader application in the assessment of patients with liver disease. Switching from 14 time points to five time points reduces patient phlebotomies by 64%, patient sampling time by 50%, and laboratory analysis and sample preparation by 64%.

The stable isotope-labelled cholates offered several unique advantages over radioisotope-labelled cholate or ursodeoxycholate used in other studies.[23, 24] First, use of stable isotopes avoids exposure of human subjects to radioactivity. Secondly, quantification by mass spectrometry–isotope ratiometry is inherently more accurate because of the added capability for compound identification. Other problems inherent with use of radiolabelled compounds, such as sequential study of intravenous and oral clearances which prolongs sampling and increases phlebotomies,[23, 24] or use of multiple radioisotopes which increases radiation exposure,[23] are avoided. With stable isotopes, because test compounds are separated by mass, multiple stable isotopes of the same compound may be administered simultaneously.

The 5-point model is generally applicable to all test compounds that exhibit certain characteristics comparable to cholate. Key characteristics include relatively high first-pass hepatic elimination, rapid and complete intestinal absorption, retention of the test compound in the intravascular space, lack of dependency on hepatic metabolism, lack of renal excretion and lack of direct effects of the test compound on the cardiovascular system or portal circulation. Cholate fulfils all of these criteria. Further validation of cholate as a appropriate test compound for assessment of portal blood flow and portal-systemic shunt is the fact that our measurement of mean cholate shunt in healthy intact human subjects (18%, or 82% first-pass hepatic elimination from the portal vein)[8] is identical to expected, based upon other studies of the liver or liver cells.

Non-invasive tests have been used for prediction of fibrosis or cirrhosis.[4] Methods include models using standard laboratory tests and other blood components,[25–29] clearance of test compounds,[19–22, 30–33] Doppler ultrasonography,[34, 35] transient ultrasonographic elastography[36–38] and magnetic resonance elastography.[39] However, none of these methods accurately quantifies the portal circulation or portal-systemic shunting. In contrast, our cholate method not only quantifies the portal circulation and portal-systemic shunting, but also correlates well with cirrhosis, varices and variceal size.

In HALT-C patients, cholate Cl_{oral} and cholate shunt compared favourably to other quantitative tests of liver function. They correlated better with cirrhosis, varices, standard laboratory tests and response to antiviral therapy than caffeine elimination, antipyrine elimination and clearance, galactose elimination capacity, MEGX generation from lidocaine and methionine breath test.[5–7] In a study of other patients, cholate Cl_{oral} and cholate shunt were better than caffeine elimination or antipyrine elimination and clearance in identifying patients at risk of future decompensation.[8]

The portal circulation may also be assessed by measurement of portal pressure. Elevation of hepatic venous portal pressure gradient (HVPG), calculated from the difference between free and wedged hepatic venous pressure, identifies patients at risk for complications of portal hypertension and may predict survival in patients with cirrhosis.[40] However, the transjugular technique for measuring HVPG is invasive, requiring percutaneous puncture of the internal jugular vein. The technique is expensive and time and labour intensive requiring procedure room, fluoroscopy, skilled medical personnel and postprocedure monitoring. Significant complications, such as carotid artery puncture, neck haematoma, venous thrombosis, pneumothorax and hemothorax, although rare, may occur in up to 1–6% of cases.[40] In our studies we have demonstrated that cholate Cl_{oral} and cholate shunt correlate strongly with prevalence of cirrhosis and varices. Advantages of the cholate method over transjugular measurement of portal pressure are the non-invasive method, lack of requirement for specialized rooms, equipment, personnel and training, and absence of any known complications, except those associated with phlebotomy from a peripheral vein. Although comparative studies are needed, characterization of the portal circulation by cholate Cl_{oral} and cholate shunt could complement or possibly replace portal pressure measurements.

We conclude that cholate Cl_{oral} and cholate shunt may be useful in the assessment of patients with chronic liver disease. The 5-point model represents a new and accurate method that reduces expenses, minimizes phlebotomy and time commitment by patients, and limits laboratory and analytical processing. These benefits could broaden application of the 5-point method for measuring cholate shunt and cholate Cl_{oral} in non-invasive assessment of the portal circulation.

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SUPPLEMENTARY MATERIAL

The following supplementary material is available for this article:

Appendix S1. Derivation of equations defining the clearance of intravenously administered cholate.

Disclosures Document. Noninvasive measurement of portal-systemic shunting in patients with fibrosis or cirrhosis due to CHC: the minimal model for measuring cholate clearances and shunt in man.

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