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Non-Invasive Assessment of Liver Function

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Abstract

Purpose of review—It is our opinion that there is an unmet need in Hepatology for a minimally- or noninvasive test of liver function and physiology. Quantitative liver function tests (QLFTs) define the severity and prognosis of liver disease by measuring the clearance of substrates whose uptake or metabolism is dependent upon liver perfusion or hepatocyte function. Substrates with high affinity hepatic transporters exhibit high “first-pass” hepatic extraction and their clearance measures hepatic perfusion. In contrast, substrates metabolized by the liver have low first-pass extraction and their clearance measures specific drug metabolizing pathways.

Recent Findings—We highlight one QLFT, the dual cholate test, and introduce the concept of a disease severity index (DSI) linked to clinical outcome that quantifies the simultaneous processes of hepatocyte uptake, clearance from the systemic circulation, clearance from the portal circulation, and portal-systemic shunting.

Summary—It is our opinion that dual cholate is a relevant test for defining disease severity, monitoring the natural course of disease progression, and quantifying the response to therapy.

Keywords

Chronic liver disease; Quantitative liver function tests; Cholate; Disease Severity Index (DSI); Cirrhosis; Hepatic Fibrosis

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Conflict of Interest

Dr. Everson is the inventor of the dual cholate test and has filed patents related to this test with the University of Colorado Denver. He is founder, manager, and equity member of HepQuant LLC and the cholate tests are HepQuant-SHUNT, HepQuant-FLOW, and HepQuant-STAT. HepQuant® is a registered trademark of HepQuant LLC and its tests. Dr. Helmke and Dr Colmenero have no conflicts to report.

Introduction

Hepatology needs an accurate, minimally- or non-invasive test of function of the whole liver. When a patient asks, “How much liver function do I have left?”, hepatologists reply with a confusing array of stage of fibrosis, blood tests, or liver stiffness. It is time for a change.

In this review we will focus on:

1. principles of quantitative liver function tests (QLFTs),
2. function - at the crossroads between fibrosis and clinical outcome,
3. quantifying the portal circulation,
4. a disease severity index (DSI) generated from the dual cholate test, and
5. potential clinical applications.

Caveats

None of the quantitative liver function tests described in this review are FDA-approved. Also, this paper should be viewed as our opinion and a position paper rather than an exhaustive review of all function tests. Although manuscripts are in preparation or submitted regarding dual cholate and DSI, most of the recent information that we discuss in this paper is from presentations at meetings or abstracts and not peer-reviewed manuscripts.

Principles of Quantitative Liver Function Tests (QLFTs)

QLFTs measure liver function using liver-specific substrates that are cleared from blood by hepatic uptake, metabolism, or both[1]. The substrates are classified as exogenous/xenobiotic (examples include indocyanine green, propranolol, nitroglycerine, antipyrine, lidocaine, midazolam, erythromycin), natural (examples include caffeine, galactose, sorbitol), or endogenous (examples include bile acids such as cholic acid, amino acids, lipoproteins). Hepatic clearance of substrates is determined by: a) liver blood flow; b) efficiency of extraction by hepatocytes; and c) metabolism by hepatocytes. Depending on the substrate and route of administration, clearance may measure or estimate hepatic blood flow, hepatic metabolism, or both [2,3]. Figure 1 displays clearance of cholate from portal and systemic circulations.

Clearance is calculated from:

$$\text{Clearance} \quad \left(mL \quad min^{-1} \quad kg^{-1}\right) = \text{Dose} \quad (mg) \,/ AUC \quad \left(mg \quad mL^{-1} \quad min \quad kg\right) \qquad \text{Equation 1}$$

where Dose is the amount of administered substrate and AUC (area under the curve) is the integral of the substrate's serum (plasma or blood) concentration versus time curve. Clearance and hepatic blood flow are related by:

$$\text{Clearance} \quad \left(ml \quad min^{-1} \quad kg^{-1}\right) = HBF \quad \left(ml \quad min^{-1} \quad kg^{-1}\right) \times \text{E} \qquad \text{Equation 2}$$

where HBF is hepatic blood flow and E is the first pass hepatic extraction of the substrate. When E=1, all of the substrate is cleared in a single pass through the liver and clearance equals HBF. If the substrate's volume of distribution is restricted to plasma volume, clearance must be adjusted by hematocrit to approximate HBF. An advantage of clearance methods is that they assess not only blood flow but also hepatic uptake or extraction efficiency; i.e., they measure effective hepatic perfusion and function.

Clearance of High Extraction Substrates

High extraction substrates are characterized by E>0.7, half lives of elimination measured in minutes, specific high-affinity hepatic transport systems, and ability to estimate hepatic blood flow [4]. Common high-extraction substrates include galactose [5], propranolol, nitroglycerine, lidocaine [6], indocyanine green (ICG) [7], sorbitol [8], and bile acids [9].

Clearance of Low Extraction Substrates

Low extraction substrates are characterized by E <0.3, and half lives of elimination of several hours to days. The clearance of a low extraction substrate reflects hepatic metabolic capacity. Changes in liver perfusion would have little effect on the clearance of substrates with metabolism-dependent routes of elimination [10]. Common substrates for measuring hepatic metabolism include phenylalanine, aminopyrine, phenacetin, methacetin, antipyrine, caffeine, diazepam, erythromycin, methionine, and α-ketoisocaproic acid [11-21].

Breath Tests

Metabolism of a substrate may also release volatile gas, such as CO_2 ,which can be measured in exhaled breath. With ^{13}C breath tests a ^{13}C label is synthetically introduced at the substrate's site of enzyme activity, so that the rate of production of $^{13}CO_2$ is related to the amount and activity of enzyme [16]. Variations in the kinetics of CO_2 may account for some variability in results, which may require administration of ^{13}C-bicarbonate to quantify and adjust for variation in CO_2 kinetics [17].

Dual Cholate Test

Cholate is an endogenous bile salt, synthesized in the liver from cholesterol, with a pool size of 1 to 3 g that is maintained by specific hepatic and intestinal transporters [3]. Cholate has a high first-pass hepatic extraction (80 to 90%), is not metabolized, and can estimate liver blood flow and perfusion [22].

Cholates have been safely administered both orally and intravenously [1,9,23,24]. In the dual test, [2,2,4,4-D]cholate (D4-cholate)is administered orally and, [24-^{13}C]cholate (13C-cholate) is administered intravenously, simultaneously. Figure 2 gives examples of the dual cholate test in health and stages of disease. The area under the serum concentration versus time curve of D4-cholate quantifies clearance from the portal circulation (Portal hepatic filtration rate (HFR)), whereas the area under the serum concentration versus time curve of 13C-cholate quantifies clearance from the systemic circulation (Systemic HFR). The ratio of 13C (IV) to D4 (oral) cholate clearance estimates portal-systemic SHUNT [24] (Equation 3).

$$SHUNT = (AUC_{D4}/AUC_{13C}) \times (Dose_{13C}/Dose_{D4}) \quad \text{Equation 3}$$

This single test quantifies three key parameters of global liver function – clearance from the systemic circulation, clearance from the portal circulation, and portal-systemic shunting.

Disease Severity Index (DSI)

Portal HFR, Systemic HFR, and SHUNT each bear a distinct relationship to fibrosis stage and clinical stages of cirrhosis. Modeling these test outputs for prediction of clinical outcomes produced a disease severity index (DSI) (Equation 4).

$$DSI = \mathrm{A}\,(SHUNT) - \mathrm{B}\,(Ln\,(Portal \quad HFR)) - \mathrm{C}\,(Ln\,(Systemic \quad HFR)) + \mathrm{D} \quad \text{Equation 4}$$

The coefficients A, B,and C and constant D were modeled from data from a long term study of patients with both early stage HCV[25] and advanced fibrosis or compensated cirrhosis (Figure 3) [24,26]. DSI quantifies global hepatic function and physiology and provides a direct link of DSI score to clinical outcome.

DSI range and cutoffs are similar in HCV disease, fatty liver disease, and cholestatic disease [25,27-31]. DSI is linearly related to biopsy-defined fibrosis stage, and has cutoffs for predicting varices, and defining risk for future outcomes. Youden Index (J) defines a test's performance in relation to its optimal cutoff value. In predicting clinical outcomes, J score was 0.59 for DSI >19, versus 0.37 for Ishak Fibrosis score >F4. The latter J score of 0.37 is similar to the J score of 0.34 reported by Forestier and colleagues for liver stiffness measurement (LSM)[32]. These results suggest that function based on DSI may outperform fibrosis based on biopsy or LSM in prediction of risk for future clinical outcomes.

Function - at the Crossroads between Fibrosis and Clinical Outcomes

Chronic liver disease, regardless of etiology, involves injury and necrosis, inflammation, fibrosis accumulation, impairment of hepatic function, and, subsequently, clinical manifestations (Figure 4). Patients without hepatic impairment have a better prognosis compared to patients with hepatic impairment. In a long term longitudinal study of cases of chronic hepatitis C, functional impairment outperformed stage of fibrosis in predicting risk for future clinical outcomes[14].

In every organ functional impairment precedes the development of clinical manifestations, symptoms and complications. Nephrologists monitor creatinine clearance, cardiologists measure ejection fraction, and pulmonologists check pulmonary functions tests to define disease severity, measure progression, and assess treatments. Hepatologists need a sensitive and reliable test to measure global liver function and physiology.

Pitfalls of the Current Clinical and Laboratory Evaluation

History and physical examination, liver enzymes, standard blood tests, imaging, elastography, and liver biopsy are insensitive to early stages of liver disease [33]. Blood levels of liver enzymes do not correlate with the severity of hepatic impairment – patients with very high ALT can have normal liver function, and patients with normal ALT can have

clinical decompensation. Conventional liver function tests, such as bilirubin, albumin, and INR, are incorporated into clinical models (MELD and CTP) which have prognostic value in end-stage liver disease[34]. But MELD score and CTP score have only been validated in patients with cirrhosis, and are not useful for measuring severity or tracking progression in pre-cirrhotic stages of disease.

PROs and CONs of Measuring Fibrosis

Measuring fibrosis by liver biopsy has been a gold standard for evaluating the patient with chronic liver disease [35,36]. However, liver biopsy is invasive, risky, anxiety-provoking, and subject to sampling error [37]. For these reasons, noninvasive methods for estimating liver fibrosis, such as elastography or serologic fibrosis markers, or a combination of both, have begun to replace staging by liver biopsy. These noninvasive alternatives can distinguish patients with cirrhosis from those without cirrhosis, but are less able to categorize the earlier fibrotic stages or later clinical stages of disease [38-40].

PROs and CONs of Measuring HVPG

Portal pressure, as measured by hepatic venous pressure gradient (HVPG), is increasingly accepted as a surrogate for clinical outcome due to the linkage of portal hypertension to varices, ascites, and encephalopathy [41,42]. However, HVPG is invasive, risky, anxiety-provoking, and can only be applied by experienced operators in specialized centers. A noninvasive alternative to HVPG is desirable. The dual cholate test is focused on the portal circulation and is potentially a noninvasive alternative to HVPG.

Quantifying the Portal Circulation

HVPG has been the traditional method for quantifying the portal circulation. But HVPG only measures pressure, is insensitive to changes occurring prior to cirrhosis, and is dampened by development of collateral vessels. Dual cholate assessed flow and hepatic uptake and can be applied over the full spectrum of alteration of the portal circulation.

Changes in the Portal Circulation at Early Stages of Disease

Despite progression of hepatic fibrosis from METAVIR F0 through F3 most patients lack signs or symptoms, standard laboratory tests are unremarkable, and HVPG is normal. Evolving pathophysiologic changes during these earlier stages of disease are not detected nor quantified! In contrast, the clearance of orally administered cholate (Portal HFR) declines by 50% or more from fibrosis stages F0 to F3 [25,26]. Based on the known relationships shown in Equation 5, the normal portal pressure and decreased Portal HFR likely reflect an increase in intrahepatic resistance.

$$\text{Portal Pressure} = (\text{Portal Blood Flow}) \times (\text{Hepatic Resistance}) \quad \text{Equation 5}$$

Necro-inflammation and activation of peri-sinusoidal stellate and endothelial cells in association with vasoactive cytokines account for the increased intrahepatic resistance to portal inflow [43]. At this early stage of disease, cure of the underlying cause could reverse the changes in the portal circulation. This positive effect of successful treatment could be

quantified by measuring Portal HFR, but not by the less sensitive measurements of fibrosis or portal pressure.

Changes in the Portal Circulation at Later Stages of Disease

As disease progresses fibrosis accumulates, hepatic resistance increases, hepatic perfusion is further compromised, and portal hypertension and portal-systemic collaterals develop [41]. Nearly all complications of chronic liver disease (ascites, varices, encephalopathy) are due to these alterations of the portal circulation. At this stage HVPG correlates with risk for decompensation and mortality [42]. But, HVPG is invasive and not embraced by patients. And, with emergence of portal-systemic collaterals the rise in HVPG is dampened. Surrogates for HVPG, such as imaging methods or liver stiffness, correlate with but do not directly measure or quantify the portal circulation or portal hypertension [44-50]. Thus, there is need for a minimally-invasive technique to quantify the portal circulation even at late stages of disease. Unlike other tests, including other QLFTs, the dual cholate test is focused on the portal circulation and may satisfy this need.

Clinical Applications

The ability to measure functional and physiological changes over the full spectrum of disease has several advantages in characterizing disease severity, monitoring progression, or measuring therapeutic effects.

Defining and Monitoring Early Stage Disease

A goal of treatment is to prevent cirrhosis by treating patients at pre-cirrhotic stages of disease. However, every currently available method for measuring disease severity has inadequate accuracy in early stages of liver disease. Current methods are unable to monitor natural progression or quantify effectiveness of treatments or interventions. In contrast, DSI has correlated linearly with stage of fibrosis, from ISHAK fibrosis stages F0 to F6 [27,28]. A comparison of the relationships of DSI, transient elastography, HVPG, and quantitative collagen to Ishak fibrosis score is shown in Figure 5 [27,28,51-54]. Only DSI demonstrates stage to stage differentiation over the full range of Ishak fibrosis scores and most notably from Ishak F0 to F4. We conclude that dual cholate and DSI is uniquely able to monitor therapeutic effects in early-stage disease.

Steatosis

Chronic hepatitis C, nonalcoholic fatty liver disease, and alcoholic liver disease are associated with hepatic steatosis which can lead to inflammation and fibrosis. Unlike imaging and elastography, DSI is not influenced by steatosis (unpublished data, presented at AASLD Emerging Trends Conference on Antifibrotic Drug Trials: Strategies and Endpoints in Chicago in June 2014).

Late Stage Disease – Diagnosing Cirrhosis

Is there a need for a QLFT to diagnose cirrhosis? Transient elastography has been approved by the FDA and is the leading noninvasive alternative to biopsy for diagnosis of cirrhosis [39,47-50,55]. But transient elastography (or magnetic resonance elastography) may

not be accurate in patients with high BMI or hepatic steatosis. Elastography may also be influenced by hepatic inflammation or blood flow, and the equipment for test performance is concentrated in liver centers and otherwise not widely available. Other noninvasive alternatives, such as serum biomarkers or clinical and laboratory models, are less accurate.

The dual cholate test outperformed a battery of metabolic tests in predicting cirrhosis [14,24,26], and DSI>19 predicted cirrhosis and risk for future clinical outcomes [27,28]. D4-cholate concentration >1μM at 60 minutes after oral dosing, is equivalent to DSI >19 [27,28]. A test requiring only one 60 minute serum sample could offer simplicity, wide-spread application, and cost savings over biopsy, elastography, and other noninvasive alternatives.

Late Stage Disease – Predicting Risk for Future Complications

The real issue is not whether a QLFT can diagnose cirrhosis, but whether function outperforms fibrosis in establishing prognosis. In the HALT-C cohort we found that the dual cholate test outperformed standard lab tests, Ishak fibrosis score, and other QLFTs in prediction of outcomes [14,24,26]. Subsequently, we compared the performance of DSI against Ishak fibrosis score, platelet count, and MELD score first by examination of respective ROC curves. The optimum cutoffs were Ishak fibrosis score > F4, MELD > 6, and platelets <150 × 10^9/L. The performance of DSI in predicting risk for future outcomes was better than these other measurements[27,28] .

Identifying the Patient with Varices

Esophageal varices are one of the more dangerous complications of liver disease. Detecting and treating the medium to large varices prior to their rupture and hemorrhage is desirable. In the HALT-C trial cholate shunt >35% or DSI>19 identified nearly all of the patients with medium- or large-sized varices [14,24,26], and DSI was more predictive than fibrosis stage [27,28] (Youden Index, J, was 0.51 for DSI versus 0.38 for Ishak fibrosis score).

Measuring Hepatic Improvement with HCV Treatment

Clearing HCV heals the liver. HCV RNA is undetectable within 1 to 4 weeks of treatment initiation with current multi-DAA regimens. In parallel, ALT normalizes and inflammation subsides. With sustained virologic response (SVR) hepatocyte functions improve – bilirubin drops, INR drops, and albumin increases. In contrast, resolving fibrosis and improving the portal circulation and portal hypertension are less certain effects. Fibrosis resolution, if and when it occurs, is a much slower process taking many months or years.

We measured the early effects at week 4 of HCV treatment in 31 patients who either had decompensated cirrhosis (Child-Pugh B and C) or were liver transplant recipients with and without cirrhosis [56]. All patients had HCV RNA <LLOQ by Week 4 of treatment. Standard blood tests, MELD score, and CTP score did not improve. Although SHUNT did not change, HFRs and DSI improved. The significant changes in HFRs and DSI, without change in bilirubin, INR, albumin, MELD score, or CTP score, would be consistent with improvement in the hepatic microcirculation due to resolution of necroinflammation. These early responders can be identified on the basis of their baseline DSI.

The long-term impact of SVR on liver function and physiology was studied in the era of interferon-based therapy. QLFTs (caffeine, antipyrine, galactose, MEGX, dual cholate, SPECT LSS) were performed at baseline and at month 24 of followup in 24 patients after achieving SVR and in 68 nonresponders [57,58]. In general, all of the functional tests improved with SVR but the most consistent and significant improvements were in DSI, SHUNT and Portal HFR. The magnitude of the improvement in the dual cholate test was similar to the magnitude of improvement in HVPG measured in a separate study [59].

Fulminant Hepatic Failure

Early recognition of prognosis in fulminant hepatic failure defines need for liver transplantation. ICG and methacetin clearance (LIMAX® test, Breath-ID ® test) are severely impaired in patients with acetaminophen or non-acetaminophen fulminant hepatic failure [60,61]. However, there is not enough experience with these tests in this complex setting to currently endorse their widespread use.

Liver Transplantation

QLFTs may predict graft failure and development of complications [62-66], but it is not clear that QLFTs could improve recipient management or independently predict graft survival. QLFTs have quantified functional return in donors and recipients after living donor transplantation [67-69].

Conclusions and future perspectives

At the ‘AASLD Emerging Trends Conference on Antifibrotic Drug Trials: Strategies and Endpoints’ in Chicago in June 2014, it was suggested that the ideal test for defining liver disease severity, monitoring progression, and measuring the effectiveness of treatment should be:

- Reproducible
- Plausibly linked to pathogenesis of disease
- Able to assess the whole organ
- Minimally invasive and well tolerated
- Able to measure effectively all stages of disease, especially early stages
- Applicable to relevant populations
- Operator independent
- Applicable across centers.

In our opinion, the dual cholate test and DSI fulfill all of these criteria and deserve further consideration in the assessment of the patient with chronic liver disease.

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Key Points

1. Hepatology has an unmet need, a functional test that globally encompasses liver function and physiology that is also linked to clinical outcomes.
2. Quantitative liver function tests, particularly dual cholate and DSI, may fill this unmet need.
3. The dual cholate test outputs a disease severity index (DSI) that is linked to clinical outcomes.
4. DSI may be useful in defining disease severity, monitoring disease progression, and for measuring response to therapy.

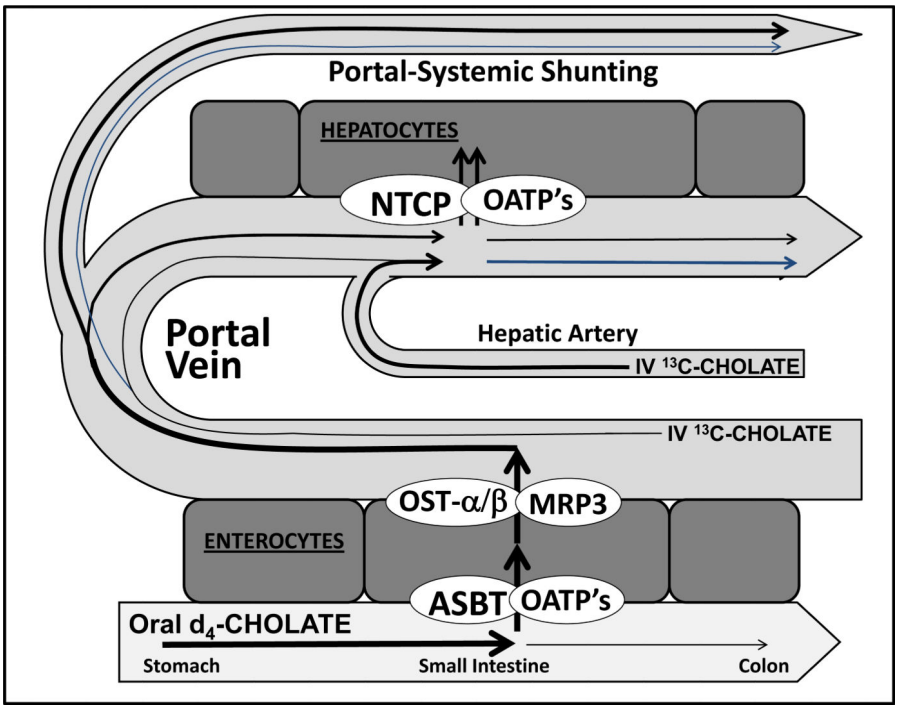

Figure 1. Delivery of intravenously and orally administered cholates to the liver for hepatic uptake

Intravenously administered cholate is delivered to the liver via both the hepatic artery and portal vein. Orally administered cholate is absorbed via the apical membrane sodium dependent bile acid transporter (ASBT) of the enterocyte. Cholate exits the enterocyte via the organic solute transporter OST-a/OST-b, enters the portal circulation, binds to albumin, and is transported to the liver sinusoids. The sinusoidal uptake of both cholates into the hepatocytes occurs predominantly at the basolateral membrane of hepatocytes, mainly by the Na-dependent taurocholate co-transporter (NTCP) and by the Na-independent superfamily of organic anion transporting polypeptides (OATP). First-pass-clearance for cholic acid is approximately 80 to 90%.

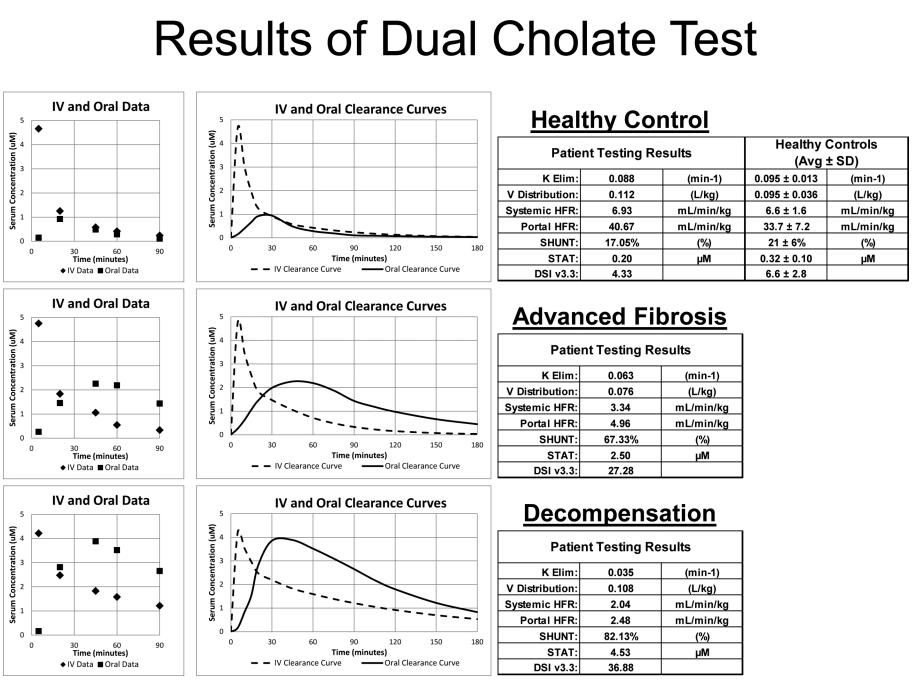

Figure 2. Individual examples of results from the Dual Cholate Test

Three examples, a healthy control (DSI 4.33), a noncirrhotic patient by fibrosis measurement but with high DSI of 27.28 and clinical outcome, and a patient with clinical complications of cirrhosis (DSI 36.88), are shown. Serum concentrations of the administered cholates are shown in the left panels, modeled clearance curves in the middle panels, and reported results in the right panels.

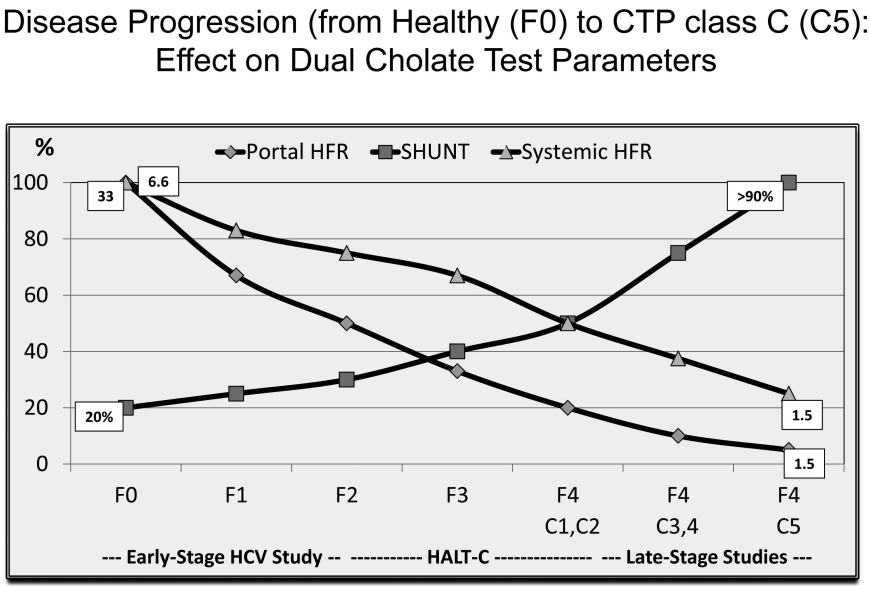

Figure 3. Model for changes in parameters of the dual cholate test from mild to end-stage liver disease

Portal HFR, Systemic HFR, and SHUNT are plotted against the stage of fibrosis (METAVIR F0 to F4) and D'Amico clinical stages of cirrhosis (C1 to C5). This plot includes data from an early stage HCV study [25], the HALT-C trial [24-26], and a mixture of studies in clinically decompensated cirrhosis. Portal HFR declines early, SHUNT increases but more dramatically in cirrhotic stages, and Systemic HFR declines later, mainly in cirrhotic stages. The different relationships of Portal HFR, Systemic HFR, and SHUNT to stages of fibrosis and cirrhosis allowed modeling of these three parameters of the dual cholate test to generate the disease severity index (DSI).

F0 to F4 represent increasing stages of METAVIR fibrosis (Poynard *et al.*, Lancet 1997). C1 to C5 represent increasing stages of cirrhosis (D'Amico *et al.*, J Hepatol 2006).

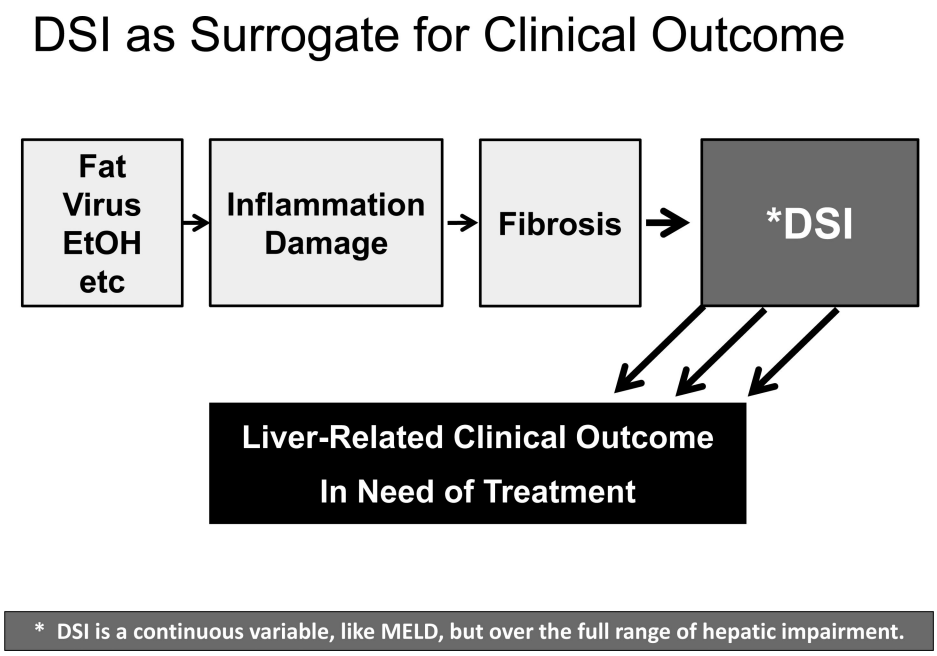

Figure 4. DSI is potentially a surrogate for clinical outcome

A functional view of the progression of liver disease would hold that etiologic agents of disease initiate damage and injury, which is sequentially followed by inflammation, fibrosis, and hepatic impairment with concomitant alteration of the portal circulation. DSI encompasses changes in both hepatic function and the altered portal circulation and is a proximal surrogate for liver-related outcomes. Because DSI and HVPG both focus on changes to the portal circulation, DSI could be viewed as potentially a noninvasive alternative to HVPG. Unlike HVPG and MELD, which only measure impairment and track changes in advanced stages of disease, DSI measures hepatic impairment over the entire spectrum of disease from F0 to F4 and through clinical stages of cirrhosis.

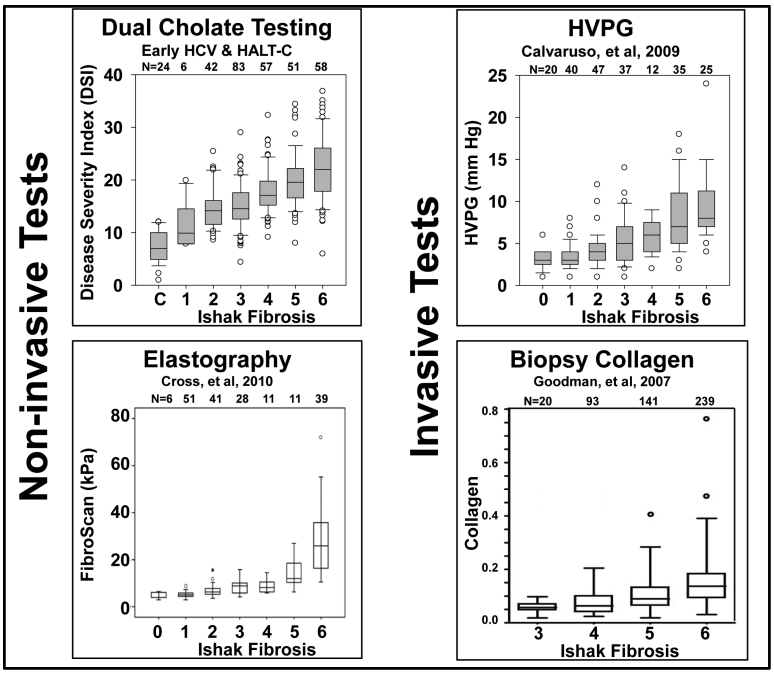

Figure 5. DSI compared to other proposed surrogates of clinical outcome

DSI[27,28], HVPG[51], transient elastography[52], and quantitative collagen from liver biopsy[53,54] are plotted against Ishak fibrosis stage (data from 4 different studies). Sample sizes are shown at the top of each plot. DSI is linear over the full range of fibrosis and, compared to the other tests, is uniquely sensitive to earlier stages of fibrosis.