Received: 21 February 2019 | Revised: 17 June 2019 | Accepted: 16 July 2019

DOI: 10.1111/chd.12831

ORIGINAL ARTICLE

Congenital Heart Disease WILEY

Assessing hepatic impairment in Fontan-associated liver disease using the HepQuant SHUNT test

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Funding information
Digestive Health Foundation Research Grant at Northwestern Memorial Hospital, Grant/Award Number: N/A

Abstract

Background & Aims: Fontan surgery for single ventricle congenital heart disease leads to Fontan-associated liver disease (FALD). Typical laboratory tests, imaging, and histopathology cannot predict clinical severity in FALD. HepQuant SHUNT is a proprietary serum test of hepatic function and physiology that has not yet been evaluated in FALD.

Methods: Fourteen adult FALD patients at a single urban tertiary care center who underwent a Fontan procedure in childhood received HepQuant SHUNT testing between September 2015 and April 2018. The HepQuant SHUNT disease severity index (DSI) assesses global liver function and physiology from systemic and portal hepatic filtration rates (HFRs, clearances adjusted for body mass) of orally and intravenously administered cholates labeled with deuterium or 13C. The SHUNT parameter of the test measures portal systemic shunting from the ratio of Systemic HFR to Portal HFR. Chart review included laboratory tests, imaging, and clinical findings. Data from FALD patients were compared with data from healthy controls.

Results: The average DSI and SHUNT values for the FALD patients were 17.5% and 36.1%, respectively, compared to 9.2% and 24.1%, respectively, for controls. Twelve (85.7%) FALD patients had a DSI >15 (upper limit of normal). Seven (50.0%) FALD patients had SHUNT values >30% (upper limit of normal), while three FALD patients (21.4%) had SHUNT values >49%. One FALD patient with preoperative SHUNT of 69%, who underwent a combined heart-liver transplant, had confirmed cirrhotic morphology within the liver explant.

Conclusions: This pilot study demonstrated that most FALD patients had hepatic impairment detected by abnormal DSI, with a smaller number having markedly elevated SHUNT values >49% suggesting intrinsic liver disease. The HepQuant SHUNT test may be useful in detecting and quantifying liver disease severity in FALD patients.

KEYWORDS

congestive hepatopathy, Fontan-associated liver disease, hepatic function tests, HepQuant SHUNT, liver transplantation

1 | INTRODUCTION

The Fontan procedure is instrumental in providing children who are born with congenital malformations that result in a single functional heart ventricle with decades of extended survival and improved quality of life. The Fontan procedure connects a single working heart ventricle to the systemic vascular system while allowing passive venous return of blood to the pulmonary arteries.[1] However, decades of exposure to passive venous congestion can lead to congestive hepatopathy, or more specifically, Fontan-associated liver disease (FALD). Unlike the inflammatory hepatopathies (eg, viral, alcoholic, and nonalcoholic fatty liver disease) which have validated biomarkers of fibrosis and clinical outcomes (eg, serum laboratory tests, fibrosis calculators, liver stiffness assessments, imaging findings, and liver biopsy), there is a growing awareness that these tools are unreliable in FALD.[2-5] Serum tests and fibrosis calculators may not be accurate to assess for fibrosis in FALD, as fibrosis deposition is not driven by inflammation, and therefore hepatic enzymes usually remain within normal limits.[3,5,6] Liver stiffness assessments are unreliable as they cannot differentiate right heart failure-induced hepatic congestion from true underlying fibrosis.[7-10] The utility of fibrosis scoring by liver biopsy has been questioned by one study demonstrating that pre-surgical liver biopsies were unreliable in predicting hepatic outcomes after heart transplantation.[5-10] Therefore, clinicians are left managing the FALD population with no validated tools to assess hepatic reserve and function.

Accurately estimating hepatic reserve and function is important to determine whether patients with a failing Fontan will require isolated heart transplantation or combined heart-liver transplantation. A combined heart-liver transplant in a patient who would have recovered normal liver function after an isolated heart transplant, or an isolated heart transplant in a patient who will soon develop decompensated cirrhosis are scenarios that cardiologists and hepatologists are working to eliminate from practice. In addition, if FALD patients have developed advanced fibrosis they require appropriate surveillance for conditions such as esophageal varices and hepatocellular carcinoma.[11,12] As a result of the lack of validated tools to assess hepatic function and reserve in FALD patients, novel methods are needed to aid clinical decision making in this poorly understood cause of liver disease.

The HepQuant SHUNT hepatic function test is based upon simultaneous measurement of cholate clearance from both systemic and portal circulations to quantify a Disease Severity Index (DSI) and portal-systemic shunting (SHUNT). In prior studies in patients with chronic hepatitis C, non-alcoholic steatohepatitis, and primary sclerosing cholangitis, DSI and SHUNT correlated with hepatic clinical complications in long-term follow-up.[13-18] Models of HepQuant SHUNT test parameters indicated that the HepQuant SHUNT test could potentially distinguish hepatic impairment due to circulatory effects from hepatic impairment due to intrinsic liver disease (see Figure S1). In the model, DSI, which is linked to total hepatic inflow, increases in proportion to either a decrease in cardiac function or decrease in hepatic function since total hepatic inflow is linked to cardiac output and hepatic cholate clearance is mainly flow dependent. In contrast to DSI, SHUNT only increases when there is disproportionate decrease in portal clearance from intrinsic liver disease or development of portal systemic shunting. Our objective in this pilot study was to evaluate the HepQuant SHUNT test in patients with FALD by correlating parameters of the HepQuant SHUNT test with other measures of liver disease.

2 | METHODS

2.1 | Patient population

This cross-sectional, proof-of-concept study was approved by the University Institutional Review Board (STU00100794-MOD0007) and carried out between September 2015 and April 2018. Patients eligible for inclusion were all post-Fontan patients 18 years or older who were evaluated by hepatology in the outpatient or inpatient setting at a single urban tertiary care center during this time frame. Patients were excluded if they were found to have alternative causes for liver disease (viral hepatitis, excessive alcohol use, etc.). Informed written consent was obtained from all participants in accordance with the Declaration of Helsinki.

2.2 | Data collection

Patient demographics and clinical characteristics (age, sex, body mass index, years since Fontan procedure, clinical signs of decompensated liver disease, diagnosis of hepatocellular carcinoma (HCC)), laboratory results (platelet count, aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, gamma-glutamyl transferase, bilirubin, international normalized ratio), imaging results (nodular appearance of the liver, presence of ascites, spleen size, lesions concerning for HCC), liver stiffness scores (Acoustic Radiation Force Impulse), ventricular function based on echocardiogram, cardiopulmonary exercise test parameters (endurance time on the Bruce protocol and maximal oxygen consumption (VO2 max)), and liver biopsy results (if available) were obtained directly from the electronic medical record from the nearest timepoint relative to the time of performance of the HepQuant SHUNT test.

The variable of ventricular function based on echocardiogram was analyzed as a binary variable, normal function or reduced function, since this was how ventricular function was reported by the interpreting cardiologist for all patients in this study (rarely was an ejection fraction specified). Initially we planned on collecting the hemodynamic variables available from cardiac catheterizations to compare with HepQuant SHUNT values, however in our patient population only 4 out of 14 patients had cardiac catheterization performed within 5 years of the HepQuant SHUNT testing. Fortunately, we did find that all but one patient received cardiopulmonary exercise testing within 1 year of the HepQuant SHUNT testing, so the variables of endurance time on the Bruce protocol and VO2 max were collected for analysis.

There were missing values as not all patients had all the above testing completed or did not have the testing completed within 1 year of the HepQuant SHUNT testing. Any missing values were

omitted from the statistical computations. Clinical outcomes (development of decompensated liver disease, discovery of HCC, organ transplantation) were ascertained through August 2018.

2.3 | HepQuant SHUNT protocol

The HepQuant SHUNT clinical testing procedure was as follows: The test compounds, cholic acid-2,2,4,4-D4 (d4-CA, product #614149) and cholic acid-24-13C (13C-CA, product #605883), were studied under FDA Investigational New Drug (IND) applications 65 123 and 65 121. Oral doses of 40 mg of d4-CA powder plus 600 mg of sodium bicarbonate powder (USP, Fisher) were supplied in plastic vials and dissolved by addition of water. Intravenous (IV) doses were formulated by Northwestern's research pharmacy. Each vial for IV use contained 22 mg 13C-cholate in 5.5 mL 8.4% sodium bicarbonate solution. Aliquots of each IV batch were analyzed for sterility and pyrogens and analyzed by HepQuant for actual final 13C-CA concentration. The HepQuant SHUNT test was performed in the morning after an overnight fast. An indwelling IV catheter was placed in an antecubital vein for administration of test compounds and collection of blood samples. The nurse administering the test thawed a vial of 13C-CA and 5.0 mL (20 mg 13C-CA) was withdrawn and mixed with 5.0 mL of 25% weight/volume human serum albumin (Plasbumin-25, Talecris). This 10 mL mixture was administered to the patient through the IV catheter over 2 minutes. The nurse administering the test added grape or apple juice to the oral d4-CA/sodium bicarbonate solution for the subject to drink at the same time as the IV injection. Peripheral venous blood samples for measurement of serum concentrations, were drawn through the indwelling catheter and obtained prior to (0 minute) and 5, 20, 45, 60, and 90 minutes after administration of cholate isotopes. Serum samples were sent to HepQuant LLC for quantification of cholate concentrations.

Serum concentrations of endogenous cholate, 13C-cholate, and d4-cholate were measured by mass spectrometry isotope ratiometry. Systemic filtration rate (hepatic clearance rate of the intravenous cholic acid normalized by body weight), portal filtration rate (hepatic clearance rate of the oral cholic acid normalized by body weight), "portal-systemic shunt fraction" (SHUNT—percentage of oral cholic acid shunted to the systemic circulation and bypassing hepatic filtration), and "disease severity index" (DSI—a proprietary score of HepQuant, ranging from 0 to 50 with 50 representing end-stage disease), were calculated from the clearances of cholate from both systemic and portal circulations. The DSI score developed by HepQuant LLC is defined from the general model:

$$\text{DSI} \approx A \times f\left[\left(B - ln\left(\text{Systemic HFR}\right)\right) + \left(C - ln\left(\text{Portal HFR}\right)\right)\right]$$

where A is a constant for scaling DSI, and B and C are limits from healthy controls.

Initial models were created from data from a long-term study of patients with chronic hepatitis C with both early stage and advanced hepatic fibrosis.[15,18-20] In subsequent studies, DSI was found to be linearly related to biopsy-defined fibrosis stage with similar clinical cutoffs that define risk for future outcomes among patients with hepatitis C, fatty liver disease, and cholestatic liver disease.[13-18] The calculated Youden Index or *J* score (defines a test's performance in relation to its optimal cutoff value) for predicting clinical outcomes was 0.59 for DSI >19, vs 0.37 for Ishak Fibrosis score >F4, suggesting increased accuracy of DSI over liver biopsy in predicting clinical outcomes in these patient populations.[18] In our study, all samples from the HepQuant SHUNT test were analyzed by HepQuant LLC without knowledge of the clinical, laboratory, or radiologic features of the subjects. The HepQuant SHUNT test results from the FALD patients were compared to the results in 30 healthy controls with normal lean body mass index values from the HepQuant LLC research trials.

2.4 | Statistics and data analysis

Univariate binomial logistic regression analysis was used to determine if DSI or SHUNT was independently associated with the categorical dependent variables of presence of ascites, presence of esophageal varices, or normal vs abnormal ventricular function as determined by echocardiogram. Univariate linear regression analysis was used to determine if DSI or SHUNT was independently associated with continuous dependent variables (eg, age, laboratory results, etc.). Pearson correlation coefficients assessed associations between DSI or SHUNT and age, time since Fontan procedure, laboratory results, spleen size, liver stiffness and cardiopulmonary exercise test parameters (endurance time on the Bruce protocol and VO2 max). A P value < .05 was considered statistically significant. Analyses were performed using SPSS Version 25.0 (Armonk, NY: IBM Corp.).

3 | RESULTS

3.1 | Patient characteristics

Of the fifteen post-Fontan patients enrolled, one patient was excluded due to chronic hepatitis C infection, leaving fourteen patients in our final cohort (The HepQuant SHUNT data and clinical data from the patient with hepatitis C are included in the Supplemental section of the article). The patients ranged in age from 20 to 50 years old (mean 35 years), and from 11 to 38 years since their Fontan procedure (mean 28.1 years). Half of the patients were males, and the average body mass index was 27.3 kg/m^2. Laboratory values, including liver enzymes, international normalized ratio (in non-anticoagulated patients), and bilirubin, were normal to only slightly elevated throughout the cohort of patients. Average platelet values were just below <150 000 × 10^9/L, and gamma-glutamyl transferase levels were slightly elevated at 115 international units per liter. Eleven (78.6%) patients had a nodular appearance of the liver on ultrasound or cross-sectional imaging, average spleen size was 14.1 cm (normal <13 cm), and average acoustic radiation force impulse was elevated at 2.7 m/s, which by most criteria in other etiologies of liver disease would be between F3 and F4 fibrosis (F4 representing cirrhosis).[21] Six patients had normal function of their dominant ventricle, seven patients had mildly reduced dominant ventricular function, and one patient had moderately reduced dominant ventricular function as

TABLE 1 Baseline laboratory values, imaging findings, liver stiffness scores, and cardiopulmonary exercise test parameters in the cohort of 14 patients with Fontan-associated liver disease

Baseline values	Mean value	SD (±)	Range
Platelet count (per 100 000 × 10^9/L)	140	55.9	47-240
Aspartate aminotransferase (units per Liter)	26	6.0	15-36
Alanine aminotransferase (units per Liter)	26	8.8	12-42
Total bilirubin (mg/dL)	1.3	1.2	0.4-4.9
Gamma-glutamyl transferase (international units per Liter)	115	66.3	35-249
International normalized ratio (excluding anticoagulation)	1.2	0.1	1.1-1.4
Albumin (g/dL)	4.7	0.3	4.2-5.0
Creatinine (mg/dL)	0.7	0.2	0.4-1.1
Spleen size (cm)	14.1*	2.6	9-19.1
Acoustic radiation force impulse (m/s)	2.7**	0.8	1.6-4.2
Endurance time on bruce protocol (minutes)	7.2	1.5	4.6-9.8
Maximal oxygen consumption—VO2 Max ((mL/(kg·min))	26.4	5.5	17-38

*Standard cutoff for splenomegaly is a craniocaudal length >13 cm.
**Cutoff values vary depending on study, but one representative study in non-Fontan-associated liver disease demonstrates reasonable cutoff values of 1.25 m/s to diagnosis F2 fibrosis and 1.75 m/s to diagnosis F4 fibrosis or cirrhosis.[21]

determined by echocardiogram. Average endurance time on Bruce Protocol was 7.2 minutes with a range of 4.6-9.8 minutes, and average Peak VO2 values were 26.4 mL/(kg min) with a range of 16.7-37.6 mL/(kg min). Mean values, standard deviations, and ranges of laboratory results, pertinent imaging findings, and acoustic radiation force impulse results are listed in Table 1.

3.2 | HepQuant SHUNT results

The HepQuant SHUNT value of DSI ranged from 9.1 to 27.6 with a mean of 17.5 in the 14 FALD patients. The HepQuant SHUNT value of SHUNT ranged from 12.0%-76.1% with a mean of 36.1%. Mean values, standard deviations and ranges for HepQuant SHUNT parameters are listed in Table 2, and DSI and SHUNT in the 14 FALD patients are graphically displayed in comparison to healthy lean controls from the HepQuant LLC database in Figure 1. A graphical representation comparing the cholate clearance curves of a Fontan patient with normal SHUNT and DSI to a Fontan patient with abnormal SHUNT and DSI is displayed in Figure 2. Unpublished data from the HepQuant LLC database indicates that lean healthy controls have an average DSI of 9.2 (SD ± 3.4) and an average SHUNT of 24.1 (SD ± 7.5). From these results suggested cutoffs from HepQuant LLC for the upper limit of the normal range are DSI 15 and SHUNT 30%.

TABLE 2 HepQuant SHUNT parameters in the cohort of 14 patients with Fontan-associated liver disease compared to healthy controls

HepQuant SHUNT parameter	Control SD (±)		Fontan SD (±)	
	Mean	SD (±)	Mean	SD (±)
Disease severity index (DSI)*	9.2	3.4	17.5	4.7
Portal-systemic shunt fraction (SHUNT) (%)**	24.1	7.5	36.1	17.7
Systemic hepatic filtration rate (ml/min/kg)	6.5	1.5	4.7	1.5
Portal hepatic filtration rate (ml/min/kg)	29.1	9.0	15.2	7.5

*Normal range for disease severity index (DSI) is <15.
**Normal range for portal-systemic shunt fraction (SHUNT) is <30%.

3.3 | Correlation between HepQuant SHUNT parameters and patient demographics, laboratory studies, imaging findings, cardiopulmonary exercise test parameters, and clinical outcomes

DSI and SHUNT values were both independently significantly associated with increasing spleen size (R = .68, P = .007 and R = .70, P = .006, respectively). Higher SHUNT was inversely associated with alanine aminotransferase values (R = −.67, P = .01), while DSI scores were not associated with alanine aminotransferase levels (R = −.17, P = .57). There was no significant association between either DSI or SHUNT values with patient age, years since Fontan operation, body mass index, aspartate aminotransferase levels, gamma-glutamyl transferase levels, platelet count, total bilirubin levels, acoustic radiation force impulse scores, the cardiopulmonary exercise test parameters of endurance time on the Bruce protocol and VO2 max, or ventricular function as determined by echocardiogram. Higher DSI and SHUNT values trended toward low platelet counts but were not statistically significant in either case (R = −.49, P = .07 and R = −.42, P = .13, respectively). DSI and SHUNT values did not significantly correlate with the presence of esophageal varices (P = .87 and P = .17,

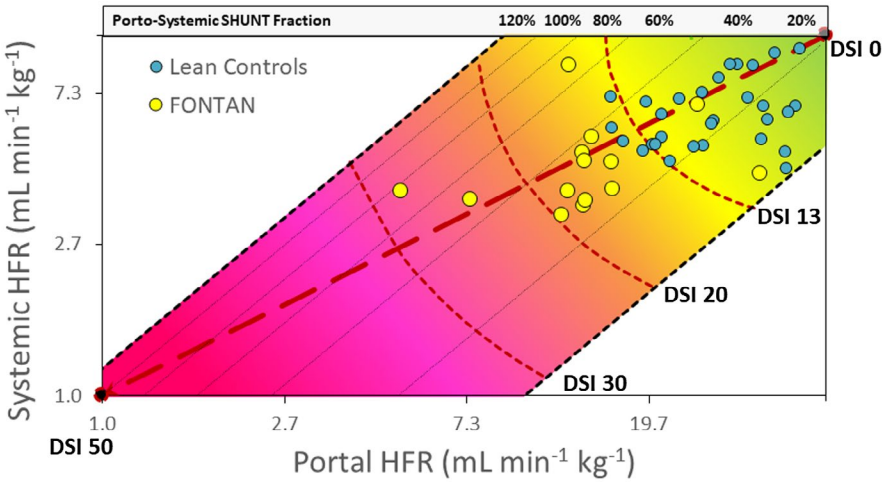
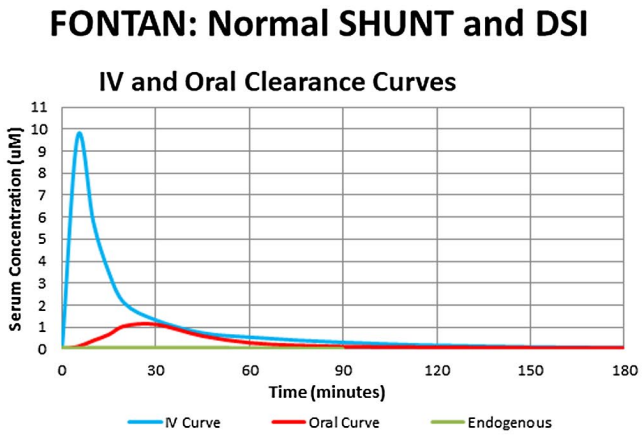
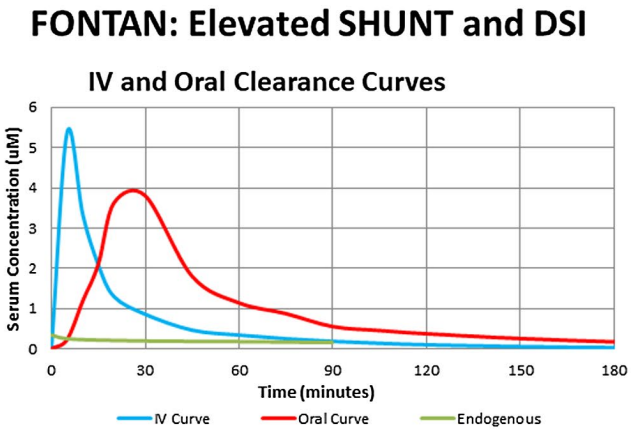


FIGURE 1 HepQuant SHUNT values in the cohort of 14 post-Fontan patients compared with lean controls
Data from lean controls (green markers) and Fontan patients (yellow markers) are overlaid onto the HepQuant Function Map. Each data point is shown in reference to portal hepatic filtration rate (Portal HFR—horizontal axis), systemic hepatic filtration rate (Systemic HFR—vertical axis), portal-systemic shunt fraction (SHUNT—dotted diagonals), and disease severity index (DSI—dashed arcs)



PATIENT TESTING RESULTS		
K Elim:	0.103	(min^{-1})
V Distribution:	0.054	(L/kg)
IV Clearance:	235	(mL/min)
Systemic HFR:	4.32	mL/min/kg
PO Clearance:	1953	(mL/min)
Portal HFR:	35.90	mL/min/kg
SHUNT:	12.0%	(%)
STAT:	0.20	µM
DSI (rtuln):	10.63	



PATIENT TESTING RESULTS		
K Elim:	0.095	(min^{-1})
V Distribution:	0.053	(L/kg)
IV Clearance:	397	(mL/min)
Systemic HFR:	3.85	mL/min/kg
PO Clearance:	522	(mL/min)
Portal HFR:	5.06	mL/min/kg
SHUNT:	76.1%	(%)
STAT:	1.57	µM
DSI (rtuln):	27.58	

FIGURE 2 HepQuant SHUNT cholate clearance curves of two cases of Fontan-associated liver disease, one with SHUNT and DSI similar to healthy controls (left plot) and the other with markedly abnormal SHUNT and DSI (right plot)

respectively) or ascites (P = .91 and P = .20, respectively). However, no patient with a SHUNT value <30% had evidence of esophageal varices, and the five patients who did have esophageal varices were all within the top six highest SHUNT values (34.2%-69.4%). P values and Pearson correlation coefficients are listed in Tables 3 and 4.

3.4 | Description of HepQuant SHUNT values and individual clinical outcomes

One patient in the cohort underwent a combined-heart liver transplant with the explanted liver pathologically confirmed to be cirrhotic. This patient had a DSI of 15.4 and a SHUNT of 69.4%. Preoperative studies showed thrombocytopenia with a platelet count of 126 000 × 10^9/L, splenomegaly with spleen size of 16.5 cm, but normal aspartate aminotransferase, alanine aminotransferase, bilirubin, and gamma-glutamyl transferase levels. The patient did have evidence of preoperative esophageal varices and ascites. Another patient had a significant hepatic complication with severe hypotension resulting in shock and multi-organ failure after a Fontan revision with postoperative hepatic encephalopathy with hyperammonemia. This patient had a DSI of 18.9 and a SHUNT of 30.6%. Preoperative studies showed thrombocytopenia with platelet count of 125 000 × 10^9/L, splenomegaly with spleen size of 15.9 cm, gamma-glutamyl transferase levels of 173 international units per

TABLE 3 Correlation of DSI and SHUNT with patient demographics, laboratory studies, and imaging findings in patients with Fontan-associated liver disease

Compared variables	*P* value**	*R* value***
Disease severity index (DSI) WITH		
Age (years)	.88	.04
Time since Fontan procedure (years)	.73	−.10
Platelet count (per 100 000 × 10^9/L)	.07	−.49
Aspartate aminotransferase (units per Liter)	.73	.11
Alanine aminotransferase (units per Liter)	.57	−.17
Total bilirubin (mg/dL)	.20	.38
Gamma-glutamyl transferase (international units per Liter)	.79	.08
Spleen size (cm)	.01*	.68*
Acoustic radiation force impulse (m/s)	.72	.12
Portal-systemic shunt fraction (SHUNT) WITH		
Age (years)	.77	−.09
Time since Fontan procedure (years)	.75	−.10
Platelet count (per 100 000 × 10^9/L)	.13	−.42
Aspartate aminotransferase (units per Liter)	.11	−.46
Alanine aminotransferase (units per Liter)	.01*	−.67*
Total bilirubin (mg/dL)	.93	.03
Gamma-glutamyl transferase (international units per Liter)	.32	−.30
Spleen size (cm)	.01*	.70*
Acoustic radiation force impulse (m/s)	.72	−.13

*Univariate binomial logistic regression analysis was used to determine if DSI or SHUNT were independently associated with the categorical dependent variables of presence of ascites or presence of esophageal varices. Univariate linear regression analysis was used to determine if DSI or SHUNT were independently associated with continuous dependent variables (eg, age, laboratory results, etc.). Pearson correlation coefficients assessed associations between DSI or SHUNT and age, time since Fontan procedure, laboratory results, spleen size, and liver stiffness. A *P* value < .05 was considered statistically significant.
**Represent values determined statistically significant by *P* value <.05.
****R* values reported represent the Pearson correlation coefficient determined through liner regression analysis.

TABLE 4 Correlation of DSI and SHUNT with cardiopulmonary exercise test parameters and ventricular function as determined by echocardiogram in patients with Fontan-associated liver disease

Compared variables	*P* value*	*R* value**
Disease severity index (DSI) WITH		
Endurance time on bruce protocol (minutes)	.64	−.15
Maximal oxygen consumption—VO2 Max ((mL/(kg·min))	.24	−.35
Ventricular function determined by echocardiogram	.93	N/A
Portal-systemic shunt fraction (SHUNT) WITH		
Endurance time on bruce protocol (minutes)	.25	−.35
Maximal oxygen consumption—VO2 Max ((mL/(kg·min))	.28	−.32
Ventricular function determined by echocardiogram	.23	NA

*Univariate binomial logistic regression analysis was used to determine if DSI or SHUNT were independently associated with the categorical dependent variables ventricular function determined by echocardiogram (either "normal" or "reduced"). Univariate linear regression analysis was used to determine if DSI or SHUNT were independently associated with the continuous dependent variables or endurance time of Bruce protocol and VO2 max. Pearson correlation coefficients assessed associations between DSI and SHUNT with continuous dependent variables. A P value < 0.05 was considered statistically significant.
***R* values reported represent the Pearson correlation coefficient determined through liner regression analysis.

liter, and total bilirubin of 1.6 mg/dL, but normal alanine aminotransferase and aspartate aminotransferase levels. The patient had evidence of preoperative ascites but not esophageal varices.

Aside from the patient who received a combined heart-liver transplant with explant confirmed cirrhosis, there were two other patients with SHUNT values >49% (49.3% and 76.1%) who also had the two highest DSI values elevated at 24.1 and 27.6, respectively. In these two cases, spleen sizes were >14 cm, platelet counts were <100 000 × 10^9/L, and bilirubin levels were >1.4 mg/dL, but both patients had normal alanine aminotransferase and aspartate aminotransferase values. These patients also had differing gamma-glutamyl transferase levels of 249 and 35 international units per liter, respectively. One of the two patients had endoscopic evidence of esophageal varices, while the other patient had radiographic evidence of ascites.

The two patients with the lowest DSI values (9.1 and 10.6) and lower SHUNT values (26.6% and 12%, respectively) had spleen sizes of 13.3 and 9 cm, platelet counts of 130 000-150 000 × 10^9/L, normal bilirubin values <1.0 mg/dL, normal alanine aminotransferase and aspartate aminotransferase values, and gamma-glutamyl transferase levels of 128 international units per liter. One patient had no evidence of clinically apparent liver disease, and the other patient had small volume ascites identified on imaging.

One patient was diagnosed shortly after HepQuant SHUNT testing with hepatocellular carcinoma, and three others had features suspicious, but non-diagnostic, for hepatocellular carcinoma on imaging. The patient with confirmed hepatocellular carcinoma had a DSI of 16.7 and a SHUNT of 24.4%. The three patients with suspicious lesions that were not confirmed hepatocellular carcinoma had DSI values of 9.1, 15.6, and 16.7, and SHUNT values of 25.6%, 29.1%, and 36.5%. Their average DSI value was 13.8 and average SHUNT value was 30.4%. Table 5 lists each of the 14 FALD patient's DSI and SHUNT values, whether each patient had findings suggestive of portal hypertension (ascites or esophageal varices), and whether each patient had a significant clinical complication.

TABLE 5 Relationship of increasing SHUNT values to DSI and findings of varices, ascites, and other clinical events in the cohort of 14 patients with Fontan-associated liver disease

Patient	SHUNT	DSI	Varices (Y/N)	Ascites (Y/N)	Other complication
1	12.0	10.63	N	N	No
2	24.4	16.72	N	N	Confirmed HCC
3	25.4	18.82	N	Y	No
4	26.2	18.46	N	N	No
5	26.6	9.10	N	Y	No
6	27.0	20.24	N	N	No
7	29.1	15.63	N	N	No
8	30.6	18.89	N	Y	Decompensation after
					Fontan revision
9	34.2	16.91	Y	Y	No
10	36.5	16.69	Y	Y	No
11	38.4	15.65	Y	N	No
12	49.3	24.15	Y	N	No
13	69.4	15.38	Y	Y	Liver explant was cirrhotic
					after heart-liver transplant
14	76.1	27.58	N	Y	No

Note: Varices were defined either endoscopically or by radiologic studies. All endoscopically defined varices were small and there were no cases of variceal hemorrhage.

4 | CONCLUSIONS

We found that the disease severity index (DSI) from the HepQuant SHUNT test was abnormal in most FALD patients, indicating that FALD patients commonly exhibit impairment of hepatic clearance of cholic acid. In contrast, the portal-systemic shunt fraction (SHUNT) in most FALD patients was within or close to the range of healthy controls (near 30%), as only three FALD patients had significantly elevated values over 49%. These findings were mostly anticipated by the model of HepQuant test parameters in the setting of cardiac disease with circulatory impairment, as described in the introduction. The model predicted that DSI, which represents total hepatic clearance, would be less specific for estimating reduced hepatic function as the DSI could be influenced by declining cardiac function in addition to hepatic impairment. In contrast, SHUNT, which represents the portal circulation, would increase with the development and progression of liver disease. A graphical representation of our pre-experimental model can be found in the Supplemental section of the article.

Increased DSI may result from decreased clearance from portal, systemic, or both circulations, and decreased clearance, in turn, may result from either decreased blood flow or decreased hepatic extraction. The molecular probe used in the HepQuant SHUNT test, cholate, is characterized by relatively high "first pass" hepatic extraction of 70%-80%; its hepatic clearance is dependent on blood flow and not metabolism. In the case of cardiac disease, diminished cardiac output could increase DSI primarily by decreasing blood flow to the liver from both portal and systemic circulations. In our FALD patients with increased DSI and normal SHUNT, we postulate the increase in DSI could be due to coordinate decrease in clearance from both portal and systemic circulations. Therefore, we would hypothesize that the decline in hepatic clearance of cholate was due to reduction in hepatic blood flow from decline in cardiac function.

In our patient population the cardiac parameters of ventricular function on echocardiogram, endurance time on the Bruce protocol, and VO2 max during cardiopulmonary stress testing did not significantly correlate with the DSI. However, Interpretation is limited by small sample size and retrospective ascertainment of these variables. For example, the echocardiogram reports only subjectively described ventricular function in a way that was difficult to discriminate cardiac function between patients (eg, ventricular function was described qualitatively as "normal or mildly reduced" versus quantitatively). In addition, although cardiopulmonary stress testing is an excellent functional tool to determine overall cardiopulmonary prognosis and cardiac reserve, it has only modest correlation with underlying hemodynamics.[22] Based on the metabolism of the cholate used in HepQuant SHUNT, we hypothesize that measured cardiac output would correlate more closely with the DSI. Unfortunately, only a small percentage of Fontan patients within our study had right heart catheterization performed so this association could not be explored. Future studies are needed to assess whether or not DSI correlates with markers of cardiopulmonary function among patients with FALD.

In contrast to DSI, SHUNT measures differential clearance from the portal circulation relative to the systemic circulation. An increased SHUNT results from increased spillover of the orally administered d4-cholate into the systemic circulation, implying intrinsic

hepatic disease, development of portal-systemic collaterals, or both. Given these physiologic principles, the three FALD patients who had significantly abnormal SHUNT values of >49% may have developed intrinsic liver disease complicating their chronic cardiac disease. This finding suggests that correction of the underlying cardiac disease might not reverse the hepatic impairment in these cases.

In support of this interpretation, the one patient in our cohort who had explant confirmed cirrhosis after a heart-liver transplant had a very elevated SHUNT of almost 70%, and the other two patients with a >49% SHUNT had evidence of either esophageal varices or ascites. In addition, the five patients in the cohort with esophageal varices were all within the top six highest SHUNT values, ranging from 34% to 69% (Table 5). These intriguing observations suggest that an additional prospective study with a larger number of Fontan cases may be warranted to further define the relationships of DSI and SHUNT to the likelihood of varices and risk for liver-related complications.

One patient who underwent a Fontan revision requiring cardiopulmonary bypass had significant intra-operative hemorrhage and prolonged hypotension resulting in acute renal and hepatic failure with hepatic encephalopathy and elevated ammonia levels. This patient had a relatively normal pre-operative SHUNT of 30% (DSI of 18.9). In this case, it is difficult to know if the patient's decompensation was a result of intrinsic liver dysfunction going into the surgery, or acute liver injury from the prolonged hypotension, or a combination of both. Given very elevated aspartate aminotransferase and alanine aminotransferase values greater than 1000 units/L postoperatively, it is likely that at least a component of liver dysfunction postoperatively was a result of acute hepatic ischemia from prolonged hypotension.

We were not surprised that there was minimal association between the DSI and SHUNT and other laboratory tests and imaging results in FALD patients outside of spleen size. Currently, no routinely performed objective measures (laboratory tests, imaging studies, liver stiffness scores, histology reports) have been proven to correlate with hepatic reserve and function in FALD. Therefore, it is reasonable to expect that novel biomarkers in FALD may not correlate with standard testing.[2-5]

An important strength of this article is that we are investigating a novel tool, the HepQuant SHUNT test, which has not been previously studied in FALD. Experimenting with new objective markers for hepatic function and reserve is needed in FALD given that regularly used objective testing has repeatedly been shown to not be predictive of disease severity. There are multiple limitations of this article, including the small sample size at a single institution. In addition, no definitive conclusions can be made from this article as to whether parameters from the HepQuant SHUNT test are predictive of clinical outcomes in FALD, such as hepatic decompensation or post-heart transplant complications. Nonetheless, the findings of this study are important for hypothesis generation.

In conclusion, in this small single center pilot study we found that FALD patients have abnormalities in hepatic function and physiology that may be detected by the HepQuant SHUNT test. Based on these preliminary results, it may be warranted to investigate the utility of the HepQuant SHUNT test, and more specifically the parameter of SHUNT, as a predictor of clinically significant hepatic disease among Fontan patients in a larger prospective study.

FINANCIAL SUPPORT AND DISCLOSURES

Dr. Lemmer and Dr. Ganger are supported by the Digestive Health Foundation Research Grant. This is an institutional grant affiliated with Northwestern University Division of Gastroenterology and Hepatology. Dr. Ganger receives a consulting fee from HepQuant LLC. No specific funding was received for this study through HepQuant LLC. Dr. Ganger is also on the speaker's bureau for Gilead and Merck and receives grant support from the NIDDK as part of the Acute Liver Failure Study Group outside the submitted work. Dr. VanWagner is supported by the National Heart, Lung and Blood Institute (K23HL136891). The views expressed in this manuscript are those of the authors and do not necessarily represent the views of the National Institutes of Health. Dr. VanWagner is on the speaker's bureau for Salix and receives research grant support from Gore Medical and Advanced Magnetic Resonance Analytics (AMRA) outside the submitted work.

ACKNOWLEDGMENTS

We would like to recognize Jeanne Gottstein who was instrumental to this project through helping with the IRB approval process, enrolling patients, and data collection.

CONFLICT OF INTEREST

Dr. Ganger receives a consulting fee from HepQuant LLC. No specific funding was received for this study through HepQuant LLC. The HepQuant SHUNT testing was paid by the institutional grant awarded to Dr. Ganger and Dr. Lemmer. Dr. Everson is formerly the Director of Hepatology at the University of Colorado Denver. In addition, he is the founder, CEO, and an equity member of HepQuant LLC. He was involved in developing the conceptual framework for using HepQuant SHUNT for assessing hepatic impairment in cardio-circulatory disease. He did not participate in drafting of the study protocol or final study design, but he did participate in data analysis and final editing of the article. Steve Helmke PhD is an employee of HepQuant LLC. He was not involved in project design, but he was involved in data analysis and final editing of the article.

AUTHOR CONTRIBUTIONS

Primary author. Responsible for drafting the manuscript, study design, data collection, data analysis, and approving the final version before submission: AL

Senior author responsible for study design and critical revisions of the manuscript and approving the final version before submission: DG

Secondary author who helped with data collection and approving the final version before submission: ZG

Secondary authors contributing to critical revisions of the manuscript and approving the final version before submission: LVW, SH, GE

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

How to cite this article: Lemmer A, VanWagner L, Gasanova Z, Helmke S, Everson GT, Ganger D. Assessing hepatic impairment in Fontan-associated liver disease using the HepQuant SHUNT test. *Congenital Heart Disease*. 2019;00:1–9. https://doi.org/10.1111/chd.12831