Published in final edited form as:

Clin Gastroenterol Hepatol. 2016 August ; 14(8): 1207–1215.e3. doi:10.1016/j.cgh.2016.03.041.

Features of Severe Liver Disease With Portal Hypertension in Patients with Cystic Fibrosis

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Abstract

Background & Aims—Liver disease is the third leading cause of death in patients with cystic fibrosis (CF), but features of patients with CF, severe liver disease, and portal hypertension have not been fully characterized.

Methods—We performed a retrospective analysis of data from 561 patients with CF (63% male, 99% with pancreatic insufficiency), liver disease (hepatic parenchymal abnormalities consistent with cirrhosis, confirmed by imaging), and portal hypertension (esophageal varices,

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Disclosures:
The authors declare no competing financial interests nor disclosures.

Authorship contributions: J.R.S., R.G.P., M.R.K., P.R.D., and S.C.L. designed the study; all authors involved in patient enrollment and data acquisition; J.R.S., C.Y.O., R.G.P., M.R.K., and S.C.L. participated in data and statistical analysis and interpretation; J.R.S., C.Y.O., M.R.K., and S.C.L. drafted the manuscript; and all authors provided critical review of the manuscript, gave their final approval, and are in agreement to be accountable for the work.

portosystemic collaterals, or splenomegaly), with no alternate causes of liver disease. All patients were enrolled in the Genetic Modifier Study of Severe CF Liver Disease at 76 international centers, from January 1999 through July 2013.

Results—Male patients were diagnosed with liver disease at a younger age than female patients (10 vs 11 years; P=.01). Splenomegaly was observed in 99% of patients and varices in 71%. Levels of liver enzymes were near normal in most patients. Thrombocytopenia affected 70% of patients and was more severe in patients with varices (88×10^9/L vs 145×10^9/L; P<.0001). Ninety-one patients received liver transplants (16%), at a median age of 13.9 years. Compared to patients who did not receive liver transplants, patients who received liver transplants had lower platelet counts (78×10^9/L vs 113×10^0/L; P<.0001), higher international normalized ratios (P<.0001), and lower levels of albumin (P=.0002). The aminotransferase to platelet ratio index (APRi) and fibrosis index based on 4 factor (FIB-4) values were above diagnostic thresholds for CF liver disease in 96% and 90% of patients, respectively. Patients who received liver transplants or who had varices had higher APRi and FIB-4 values than patients who did not.

Conclusions—In patients with CF, severe liver disease develops early in childhood (around 10 years of age) and is more common in boys than girls. Patients with varices and those who receive liver transplants have more abnormal platelet counts and APRi and FIB-4 scores.

Keywords

CFLD; ALT; phenotype; portal pressure; INR

Introduction

Cystic fibrosis (CF) is a life-limiting multisystem disease caused by mutations in both alleles of the *CFTR* gene. Liver disease is an independent risk factor for mortality[1] and the third leading cause of death in CF, accounting for an overall mortality of 2.5%. The risk of developing cirrhosis (3-5%) is likely related to non-*CFTR* genetic variation and environmental influences[2].

The non-specific term “CF-related liver disease” has been used to describe a spectrum of hepatobiliary diseases, ranging from neonatal cholestasis, liver biochemical changes, imaging abnormalities (e.g. liver parenchymal heterogeneity on ultrasound), and a variety of histological abnormalities (e.g. hepatic steatosis, cholestasis, and/or fibrosis), to severe liver disease characterized by cirrhosis with portal hypertension. In view of varying definitions previously used to report CF-related liver diseases, the phenotype of CF patients with cirrhosis and portal hypertension (i.e. severe CFLD) has not been properly characterized.

As part of our worldwide, multicenter study of genetic modifiers in CF, we carefully characterized the phenotype of the largest reported group of CF patients with severe liver disease with portal hypertension.

Methods

CFLD patients in this analysis were independently and specifically enrolled in the Genetic Modifier Study of Severe CF Liver Disease[3] from 76 international CF centers between the 14 year period from January, 1999, and July, 2013 (**Figure 1**).

Study enrollment required a CF diagnosis[4], age ≥ 2 years, and diagnosis of CFLD confirmed by 1) imaging showing hepatic parenchymal abnormalities consistent with cirrhosis (including heterogeneous liver parenchyma), and 2) evidence of portal hypertension (including esophageal varices at endoscopy, portosystemic collaterals on imaging, or splenomegaly), and 3) absence of an alternative diagnosis for liver disease or portal hypertension. We excluded patients with prolonged use of parenteral nutrition in infancy (> 2 months) and portal vein thrombosis without cirrhosis. For patients who underwent liver transplantation, clinical data were obtained prior to transplantation. Our diagnostic definition did not include liver biopsy since biopsies are not routinely performed in this population and the patchy nature of disease risks sampling error.

Data were independently reviewed by two physicians (P.R.D., S.C.L.) with CFLD expertise. Lack of agreement was resolved by discussion or with additional clinical data when necessary/available. Institutional review boards of all participating institutions approved this study.

The upper limit of normal (ULN) for males and females under 18 years old was 40 and 35 IU/L for alanine aminotransferase (ALT), and for patients over 18 years old, 30 and 20 IU/L. ULN for gamma-glutamyl transferase (GGT) was ≤ 30 IU/L[5-7]. The aspartate aminotransferase (AST) to platelet ratio index (APRi) and fibrosis index based on four factors (FIB-4) were also calculated[8-10].

Summary statistics, including counts with percentages, and medians with either range or 25^{th} and 75^{th} percentiles, were computed. Fisher's exact test or Wilcoxon rank sum test were used. p value of < 0.05 was considered statistically significant.

Results

Fifty-one of the 612 enrolled patients (8%) with a presumed diagnosis of CFLD were excluded (**Figure 1**); 32 due to an additional cause of liver disease or insufficient evidence of portal hypertension, 15 with incomplete clinical documentation, and 4 whose diagnosis of CFLD occurred > 30 years of age and therefore considered to be outliers.

We analyzed 561 patients from 76 CF centers in 24 countries (**Figure 1**, **Table 1**). Enrollment average age was 18.3 years (range 2.1 to 52.5 years), 63% male, and 94% Caucasian. Almost all CFLD patients were pancreatic insufficient (PI) (99%) and had two severe, loss-of function *CFTR* mutations (92%) (**Table 1**).

The median age of CFLD diagnosis was 10 years (**Figure 2**), 94% were diagnosed by 20 years, 80% by 15 years, and 45% before 10 years old. Males with CFLD were diagnosed earlier than females (10.2 vs 11.4 years, $p = 0.003$) (**Figure 2**).

Table 2 illustrates phenotypes and therapies in the CFLD patients. Varices were present in 71% of the patients, of whom 85% had esophageal varices (35% of whom also had gastric varices) and 15% had only gastric varices. Males had documented varices earlier than females. Liver biopsy confirmed CFLD in 35% of patients, performed at a median of 4.8 years before enrollment (range < 0.5 to > 20 years). Cholelithiasis was documented in 8% and distal intestinal obstruction syndrome (DIOS) in 26% of patients > 1 year old. Meconium ileus (MI) at birth occurred in both genders at similar rates (**Table 2**).

Most CFLD patients had liver enzyme concentrations in the normal to <2X ULN range (63%). GGT was more commonly elevated than ALT. Bilirubin concentrations were normal or <2X ULN in most patients (**Table 3**). Males had significantly higher median GGT, INR, and albumin concentrations than females (**Table 3**).

We calculated APRi and FIB-4 in 497 CFLD patients and found values differed by age and male gender, largely due to differences in AST (**Table 4**). APRi and FIB-4 scores exceeded the diagnostic thresholds reported by Leung et al. (APRi >0.264 and FIB-4 >0.358)[10] in 96% and 90% of our patients, respectively. Additionally, these biomarkers were significantly different in CFLD patients with and without known varices as well as those who have and have not undergone liver transplantation **(Table 4).**

Thrombocytopenia was common overall (70%), and more prevalent among patients with varices (78% vs 55%) whose platelet counts were significantly lower than those patients whose status of varices was unknown (median 88×10^9/L vs 145×10^9/L; $p < 0.0001$) **(Table 3).** Patients with a transjugular intrahepatic portosystemic shunt (TIPS; n=40) were removed from analyses of platelet count to avoid confounding by shunt-induced changes to the platelet count. Patients with varices and also those undergoing liver transplantation were characterized by greater abnormalities in markers of disease severity (**Tables 2 and 3**).

Therapies for CFLD are summarized in **Table 2**. Among 91 liver transplant recipients (16%), there were no significant differences between gender and age in the proportion transplanted. Fifteen subjects (3%) underwent lung transplantation, 10 of whom underwent both lung and liver transplantation with eight patients having the transplants simultaneously **(Table 2)**. Before transplantation, patients receiving a liver transplant had significantly lower platelet count (median platelet count 78×10^9/L vs 113×10^9/L; $p < 0.0001$) and were more likely to have elevated INR (INR >1.5 in 38% vs 12%, $p < 0.0001$) and lower albumin (albumin <3.5 g/dL in 60% vs 37%; $p = 0.0002$) compared to patients not undergoing liver transplantation (**Table 3**).

Discussion

This worldwide study of 561 patients represents the largest reported cohort of CFLD patients enrolled according to a strict definition of severe disease and presents, for the first time, a rigorous and robust description of the characteristics of patients with severe CFLD with portal hypertension. The impact of liver disease on the health of patients with CF appears to be limited primarily to those with severe disease with portal hypertension. However, many published studies include a broader group of patients and do not adequately

distinguish descriptions of those with cirrhosis from those with milder disease. A clear understanding of the phenotype of severe CFLD enables comparison between patient cohorts and between different studies. Our description provides an important benchmark that is widely generalizable because it is derived from a large multi-national sample.

Key observations in this study include that severe CFLD: (a) arises early in life (median 10y) and new cases rarely appear in adulthood; (b) occurs in pancreatic insufficient patients (99%) or in patients who had two severe, loss-of function *CFTR* mutations (92%), which is considerably higher than the prevalence of pancreatic insufficient mutations in the general CF population (85%)[11]; (c) occurs in males more frequently than females (63% vs 37%); (d) is associated with normal or mildly elevated liver enzymes and liver function tests, and having an APRi >0.264[10] and FIB-4 >0.358[10] in the majority of patients, and (e) causes more severe abnormality in bloodwork markers of disease severity in those patients with varices and in those requiring liver transplantation (i.e. platelets, albumin, INR, and total bilirubin). Our findings are consistent with some previous reports based on smaller, single-center studies of CF patients with varying definitions of liver disease that were not necessarily limited to cirrhosis and portal hypertension[12,13].

Previous studies of MI in CFLD have shown conflicting results[14], which may arise from the effects of small patient populations, varying definitions of MI (e.g. including meconium ileus “equivalent” or DIOS), and/or varying definitions of CF liver disease. The prevalence of MI in the global CF population is approximately 15-20%, but 17-22% in those with pancreatic insufficiency[15]. Nearly all of our CFLD patients have pancreatic insufficient CF and the incidence of MI in our patients is 22%, which is therefore not different from the appropriate control CF population without CFLD, suggesting that MI at birth does not increase the likelihood of severe CFLD. In addition, MI was not different when comparing patients with and without liver transplantation, nor those with or without known varices.

CFLD with portal hypertension affects males more commonly than females, even though it appears to develop before puberty in the majority of cases. Gender differences in prevalence or severity have also been described in primary sclerosing cholangitis, autoimmune hepatitis, viral hepatitis, non-alcoholic fatty liver disease, hepatocellular carcinoma, and primary biliary cirrhosis. Proposed explanations include differential exposure to environmental, hormonal, immune, and genetic factors. Gender differences have been demonstrated in hepatic gene expression[16], hepatic function[17], and immune function[18]. Studies have yet to explore these complex interactions in CFLD.

There was no pattern of liver enzyme abnormality that characterized these patients with severe CFLD or that would enable them to be readily differentiated from patients with milder variants of liver disease. Liver enzyme abnormalities are highly prevalent among all patients with CF, regardless of liver disease status[19]. Low platelet count was common in our cirrhotic CFLD patients and was more pronounced in those with varices and those undergoing transplantation, suggesting a role for this variable in diagnosis of severe CFLD and as a marker of disease severity. Future studies of platelet count changes in individual patients over time are awaited. Median INR was statistically significantly higher in patients

with varices and in those undergoing liver transplantation, but the absolute numerical differences were small and therefore of uncertain clinical usefulness.

The failure of standard blood tests and imaging to differentiate patients with CFLD highlights the need for the development of novel biomarkers for this important disease. Recent studies have tested the diagnostic accuracy of transient elastography, proteomic profiling, and a panel of circulating microRNAs[20-22]. The role of serum markers of fibrogenesis has also been studied[23]. These initial studies demonstrate that differences between established CF liver disease and CF patients with absence of liver disease can be shown. Based on a different definition of CFLD, APRi and FIB-4 were recently shown to predict CFLD with moderate sensitivity and specificity[10]. In nine of their CF patients with portal hypertension (which is the most comparable population to our 497 CF patients), they reported APRi and FIB-4 values of 0.96 and 0.76, respectively, compared to our median values of 1.15 and 1.16. Future studies may investigate the role of other non-invasive tests in CFLD, such as a “clinical prediction rule” (calculated from platelet count, spleen size z-score, and albumin concentration) that is validated for the non-invasive diagnosis of children with esophageal varices[24]. There is a pressing need for further research in this area, particularly to identify markers that may predict children who will develop severe liver disease at an early time-point when preventative therapies may be effective.

The majority of CFLD patients in this study (89%) were receiving ursodeoxycholic acid, consistent with published recommendations[25]. Nonetheless, there is no evidence that this therapy positively modifies the clinical course of CFLD[26] and the potential risk of adverse outcomes in patients with CFLD has not yet been adequately explored[27,28]. There remains a need for studies to elucidate the relative risks and benefits of ursodeoxycholic acid therapy for CFLD.

Sixteen percent of our patients have undergone liver transplantation. In general, among patients with CFLD severe enough to be listed for liver transplantation, there is a survival benefit for patients who achieve transplant[29]. Although criteria for listing for liver transplantation for CFLD have been suggested[25], it is often challenging to determine the optimal timing of liver transplantation in cirrhotic CFLD patients. It is hoped that future prospective studies may help to further clarify the indications and optimal timing of liver transplantation for severe CFLD.

Our study is the largest population of well-phenotyped CF patients with portal hypertension due to cirrhosis. The limitations of this study include the lack of availability of requested data for some subjects, and lack of assessment of differences in clinical practice among the 76 CF centers (e.g. dose of ursodeoxycholic acid, indications for endoscopy, liver biopsy, and liver transplantation). We were not able to measure the prevalence of disease due to the wide variation between enrolling centers in their referral patterns and ability to accurately characterize the population of CF patients that they serve. Longitudinal data in individual patients was not collected. However, recruitment of subjects worldwide ensures generalizability of our findings across all CF populations.

In summary, our international collaboration has successfully compiled and described the clinical characteristics of the largest reported group of adults and children with severe CFLD. We have confirmed that this disease presents early in childhood, males are more likely to be affected, liver enzymes are usually normal or near-normal, and nearly all have pancreatic insufficiency. Patients with varices, as well as those who require liver transplantation, are characterized by greater abnormalities of platelet count, albumin, INR, total bilirubin, APRi and FIB-4. Further studies with this group of patients are underway, aiming to define further genetic risk factors and pathways to help our understanding of disease pathogenesis and to identify potential therapeutic targets.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

We are indebted to the contributing CF centers and their principal investigators and study coordinators for patient enrollment into the Genetic Modifier Study of Severe Cystic Fibrosis Liver Disease who were acknowledged previously[3] (see Supplementary Material), and more recent enrolling CF Centers: *Colorado*: M. Narkewicz, S. Burr; *Indiana*: J. Molleston, E. Byam; *Kansas*: D. Polineni, L. Scott; *Mississippi*: J. Majure, M. King; *Pennsylvania*: L. Varlotta, M. Aramburo; *Australia*: C. Ooi, L. Nahidi, K. Gaskin, K. McKay; *Belgium*: K. De Boeck, N. Feyaerts; *Estonia*: M. Vasar; *France*: H. Corvol, P. Touche; *Hungary*: R. Ujhelyi, A. Szabo; *Italy*: V. Lucidi, F. Alghisi; *Lithuania*: S. Dumcius; *Macedonia*: S. Fustik; *Poland*: J. Walkowiak, M. Dus-Zuchowska; *Serbia*: P. Minic, N. Savic; *Sweden*: A. Lindblad, K. Strandner; Ukraine: H. Makukh; *United Kingdom*: D. Kelly, I. Van Mourik, C. Lloyd.

The authors thank The University of North Carolina Center for Bioinformatics (Drs. Hemant Kelkar and Annie Xu) and The University of North Carolina DNA Laboratory for their contributions. Lastly, the authors thank Dr. Dominique Debray and Pauline Touche for their assistance with French patient recruitment, enrollment, and collection of clinical data.

Grant Support:

This work was supported by grants from the Cystic Fibrosis Foundation for J.R.S. (STONEB12I0) and M.R.K. (KNOWLE00A0), from the NIH for M.R.K. (DK066368; HL068890), in France for H.C. from ANR (R09186DS), DGS, VLM, and AICM, and in Canada for P.R.D. from CF Canada, Genome Canada, Ontario Research Fund, and Lloyd Harris Carr Foundation.

List of abbreviations

ALT	alanine transferase
APRi	aspartate aminotransferase to platelet ratio index
AST	aspartate aminotransferase
CF	cystic fibrosis
CFLD	cystic fibrosis liver disease
DIOS	distal intestinal obstruction syndrome
FIB-4	fibrosis index based on 4 factors
GGT	gamma-glutamyl transferase

INR	international normalized ratio
IU/L	international units per liter
MI	meconium ileus
PI	pancreatic insufficient
TIPS	transjugular intrahepatic portosystemic shunt
ULN	upper limit of normal

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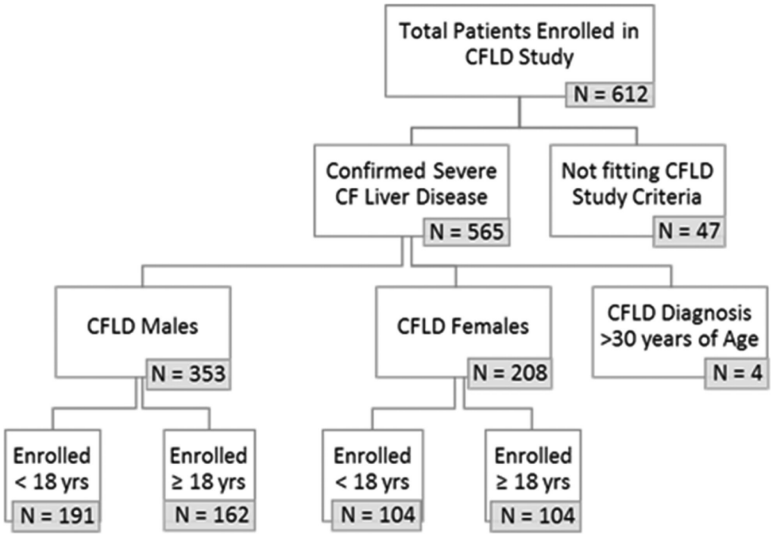

Fig. 1. Patients enrolled in the Genetic Modifier Study of CF Liver Disease (CFLD Study) from January, 1999 up to July 2013, by age and gender

Those who were confirmed with severe CFLD and diagnosed after 30 years (n=4) were four or more standard deviations above the mean of the normal distribution, which more likely reflects an environmental insult, and therefore were not included in the analysis.

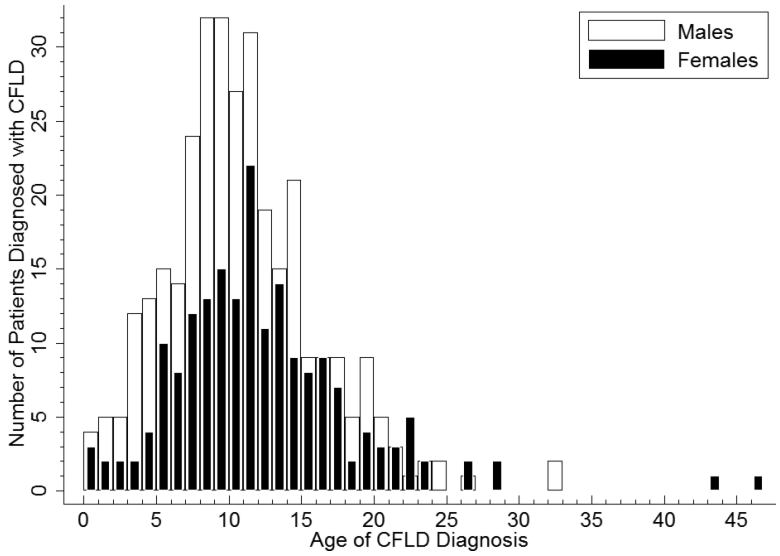

Fig. 2. Age of liver disease diagnosis in CFLD subjects by gender

Age distribution of diagnosis of liver disease (portal hypertension) in patients with confirmed CFLD and a reported year of CFLD diagnosis (n=515). Males in white bars and females overlapped in black bars.

Table 1

Demographics and characteristics in CFLD patients

Description	All *n* =561	Males[a] *n* =353 (63%)	Females *n* =208 (37%)
Age (years) at enrollment, mean (±SD)	18.3 (±7.2)	18.1 (±6.8)	18.7 (±7.8)
Median (range)	17.4 (2.1-52.5)	17.2 (2.8-44.8)	17.9 (2.1-52.5)
Race, *n* (%)			
Caucasian	529 (94)	334 (95)	195 (94)
Hispanic	16 (3)	8 (2)	8 (4)
African American	4 (1)	2 (1)	2 (1)
Other	12 (2)	9 (2)	3 (1)
Enrollment by continent, *n* (%)			
North America	273 (49)	166 (47)	107 (52)
Europe	242 (43)	158 (45)	84 (40)
Australia	25 (5)	17 (5)	8 (4)
Asia	13 (2)	8 (2)	5 (2)
South America	8 (1)	4 (1)	4 (2)
Pancreatic status, *n* (%)			
Pancreatic insufficient (PI)	556 (99)	351 (99)	205 (99)
Pancreatic sufficient (PS)	5 (1)	2 (1)	3 (1)
PI/PS genotype based on *CFTR* alleles, *n* (%)			
PI/PI	514 (92)	324 (92)	190 (92)
PI/PS	11 (2)	6 (2)	5 (2)
PI/Unknown	28 (5)	20 (5)	8 (4)
Unknown/unknown	8 (1)	3 (1)	5 (2)
Pancreatic insufficiency prevalence, *n* (%)	*n* =486	*n* =311	*n* =175
0.75 – 1.0 (severe)	473 (97)	303 (97)	170 (97)
0.25 – < 0.75 (moderate)	12 (2)	8 (3)	4 (2)
< 0.25 (mild)	1 (1)	0 (0)	1 (1)
CFTR genotype available, *n* (%)	*n* =525	*n* =330	*n* =195
Phe508del/Phe508del	298 (57)	196 (59)	102 (52)
Phe508del/Other	183 (35)	106 (32)	77 (40)
Other/Other	44 (8)	28 (9)	16 (8)

[b] Pancreatic insufficiency prevalence (PIP) score developed and validated by Ooi and Durie[11].

[a] CFLD occurs in males more frequently than females ($p = 0.0006$).

Table 2

Liver disease phenotypes and therapies in CFLD patients

Description	All *n*=561	Males *n*=353	Females *n* =208	*p* value	No known varices *n* =54	Varices present *n* =384	*p* value	No liver transplant *n* =470	Liver transplant *n* =91	*p* value
Splenomegaly										
Data available	*n* =554	*n* =350	*n* =204		*n* =153	*n* =383		*n* =467	*n* =87	
Splenomegaly present, *n* (%)	548 (99)	346 (99)	202 (99)	1.00	148 (97)	382 (99)	0.84	461 (99)	87 (100)	1.00
Varices										
Data available	*n* =538	*n* =339	*n* =199		*n* =154	*n* =384		*n* =452	*n* =86	
Varices present, *n* (%)	384 (71)	241 (71)	143 (72)	0.94	0 (0)	384 (100)	**<0.0001**	308 (68)	76 (88)	0.14
Median age (range) years	12.6 (0.1-31.0)	12.4 (2.4-31.0)	13.7 (0.1-29.8)	**0.02**	not applicable	12.7 (2.7-31.0)	not applicable	12.9 (0.1-31.0)	11.6 (2.5-27.5)	**0.03**
Liver biopsy										
Data available	*n* =539	*n* =338	*n* =201		*n* =146	*n* =373		*n* =457	*n* =82	
Liver biopsy present, *n* (%)	191 (35)	118 (35)	73 (37)	0.86	45 (31)	137 (37)	0.39	149 (33)	42 (51)	**0.04**
Cholelithiasis										
Data available	*n* =545	*n* =344	*n* =201		*n* =150	*n* =377		*n* =461	*n* =84	
Cholelithiasis present, *n* (%)	46 (8)	29 (8)	17 (8)	1.00	11 (7)	34 (9)	0.73	40 (9)	6 (7)	0.83
Distal intestinal obstruction syndrome (DIOS)										
Data available	*n* =539	*n* =337	*n* =202		*n* =149	*n* =367		*n* =453	*n* =86	
DIOS present, *n* (%)	138 (26)	80 (24)	58 (29)	0.33	31 (21)	100 (27)	0.28	111 (25)	27 (31)	0.31
Meconium Ileus (MI)										
Data available	*n* =560	*n* =352	*n* =208		*n* =153	*n* =384		*n* =469	*n* =91	
MI present at birth, *n* (%)	125 (22)	76 (22)	49 (24)	0.68	36 (24)	86 (22)	0.83	101 (22)	24 (26)	0.43
Ursodeoxycholic acid (URSO)										
Data available	*n* =446	*n* =276	*n* =170		*n* =125	*n* =311		*n* =382	*n* =64	
URSO, *n* (%)	399 (89)	247 (89)	152 (89)	1.00	107 (86)	283 (91)	0.70	344 (90)	55 (86)	0.84
Transjugular intrahepatic portosystemic shunt (TIPS)										
Data available	*n* =545	*n* =344	*n* =201		*n* =149	*n* =348		*n* =461	*n* =84	
TIPS present, *n* (%)	47 (9)	28 (8)	19 (9)	0.64	5 (3)	40 (11)	**0.006**	37 (8)	10 (12)	0.30

Description	All *n*=561	Males *n*=353	Females *n* =208	*p* value	No known varices *n* =54	Varices present *n* =384	*p* value	No liver transplant *n* =470	Liver transplant *n* =91	*p* value
Median age (range) years	15.3 (7.8-30.5)	13.5 (7.8-30.4)	15.5 (9.8-21.4)	0.38	10.9 (7.8-18.1)	15.4 (8.2-30.4)	0.17	15.5 (7.8-30.4)	14.4 (8.2-21.2)	0.23
Organ transplant										
Liver transplant										
Data available	*n* =561	*n* =353	*n* =208		*n* =154	*n* =384		*n* =470	*n* =91	
Liver transplant present, *n* (%)	91 (16)	59 (17)	32 (15)	0.81	10 (7)	76 (20)	**0.0006**	0 (0)	91 (100)	**<0.0001**
Median age (range) years	13.9 (0.6-33.6)	13.8 (0.6-30.0)	14.2 (0.8-33.6)	0.35	14.9 (8.0-21.6)	13.9 (0.6-30.9)	0.94	not applicable	13.9 (0.6-33.6)	not applicable
Lung transplant										
Data available	*n* =561	*n* =353	*n* =208		*n* =151	*n* =382		*n* =470	*n* =91	
Lung transplant present, *n* (%)	15 (3)	9 (3)	6 (3)	0.79	0 (0)	13 (3)		5 (1)	10 (11)	**<0.0001**
Median age (range) years	25.7 (13.0-35.2)	25.7 (13.0-35.2)	25.1 (13.4-30.9)	0.91	not applicable	25.7 (13.4-35.2)	**0.02**	24.7 (13.4-33.3)	26.1 (13.0-35.2)	0.81
Liver & lung transplant (subset of above)										
Data available	*n* =561	*n* =353	*n* =208		*n* =151	*n* =382		*n* =470	*n* =91	
Liver & lung transplant present, *n* (%)	10 (2)	6 (2)	4 (2)	1.00	0 (0)	8 (2)	0.11	0 (0)	10 (11)	**<0.0001**
Median age (range) years	24.5 (13.0-30.9)[a]	25.7 (13.0-30.0)	23.2 (21.5-30.9)	0.29	not applicable	25.7 (21.5-30.9)[b]	not applicable	not applicable	24.5 (13.0-30.9)[a]	not applicable

p values (<0.05 shown in bold) generated using Fisher's exact test (for categorical data) or Wilcoxon rank sum (for medians).

[a]Median age provided for 8 patients with simultaneous liver and lung transplants; one male and one female patient had transplants separated by 7 and 21 years, respectively, and are not included in the median calculation

[b]Median age provided for 7 patients with simultaneous liver and lung transplants; one male patient had transplants separated by 21 years, and is not included in the median calculation.

Table 3

Laboratory values by gender and phenotype in CFLD patients

Description	All *n* =561	Males *n* =353	Females *n* =208	*p* value	No known varices *n* =154	Varices present *n* =384	*p* value	No liver transplant *n* =470	Liver transplant *n* =91	*p* value
Male gender, *n* (%)	353 (63)			**0.0006**	98 (64)	241 (63)	0.94	294 (63)	59 (65)	0.85
Alanine aminotransferase (ALT), IU/L	*n* =532	*n* =334	*n* =198		*n* =150	*n* =365		*n* =454	*n*=78	
Median (25^{th}, 75^{th})	41 (28, 67)	44 (28, 71)	38 (26, 36)	**0.01**[a]	46 (31, 68)	40 (27, 66)	0.09	42 (28, 68)	39 (27, 59)	0.56
Gamma-glutamyl transferase (GGT), IU/L	*n* =456	*n* =295	*n* =161		*n* =128	*n* =316		*n* =390	*n* =66	
Median (25^{th}, 75^{th})	60 (30, 123)	66 (33, 127)	51 (25, 113)	**0.04**	63 (30, 106)	57 (30, 126)	0.96	61 (31, 123)	52 (25, 134)	0.83
Total bilirubin, mg/dL	*n* =474	*n* =292	*n* =182		*n* =119	*n* =341		*n* =394	*n* =80	
Median (25^{th}, 75^{th})	0.8 (0.5, 1.3)	0.8 (0.5, 1.3)	0.8 (0.5, 1.4)	0.74	0.7 (0.4, 1.0)	0.9 (0.5, 1.5)	**0.0001**	0.8 (0.5, 1.2)	1.0 (0.7, 1.6)	**0.001**
Direct bilirubin, mg/dL	*n* =231	*n* =143	*n* =88		*n* =53	*n* =1 70		*n* =194	*n* =37	
Median (25^{th}, 75^{th})	0.3 (0.2, 0.6)	0.3 (0.2, 0.6)	0.3 (0.2, 0.6)	0.66	0.2 (0.1, 0.4)	0.4 (0.2, 0.7)	**0.0001**	0.3 (0.2, 0.5)	0.4 (0.3, 0.8)	**0.01**
Albumin, g/dL	*n* =493	*n* =306	*n* =187		*n* =138	*n* =342		*n* =418	*n* =75	
Median (25^{th}, 75^{th})	3.6 (3.2, 4.0)	3.7 (3.3, 4.0)	3.5 (3.0, 3.9)	**0.0007**	3.8 (3.3, 4.1)	3.5 (3.1, 3.9)	**0.001**	3.7 (3.2, 4.0)	3.3 (2.9, 3.8)	**0.0002**
International normalized ratio (INR)	*n* =360	*n* =223	*n* =137		*n* =100	*n* =249		*n* =310	*n* =50	
Median (25^{th}, 75^{th})	1.2 (1.1, 1.4)	1.3 (1.1, 1.4)	1.2 (1.1, 1.3)	**0.03**	1.2 (1.1, 1.3)	1.3 (1.1, 1.4)	**<0.0001**	1.2 (1.1, 1.4)	1.4 (1.2, 1.7)	**<0.0001**
Platelet count ($\times 10^9$/L)	*n* =464	*n* =290	*n* =174		*n* =134	*n* =313		*n* =402	*n* =62	
Median (25^{th}, 75^{th})	103 (65, 166)	101 (62, 160)	111 (69, 172)	0.34	145 (87, 198)	88 (56, 142)	**<0.0001**	113 (70, 171)	78 (54, 99)	**<0.0001**
< 150, *n* (%)	324 (70)	208 (72)	116 (67)	0.65	74 (55)	243 (78)	**0.048**	269 (67)	55 (89)	0.19
< 75, *n* (%)	140 (30)	91 (31)	49 (28)	0.62	21 (16)	117 (37)	**0.0006**	113 (28)	27 (44)	0.10

p values (<0.05 shown in bold) generated using Fisher's exact test (for categorical data) or Wilcoxon rank sum (for medians).

Data presented as median with 25^{th} and 75th percentiles or frequency with percent.

[a]Apparent difference between genders is clinically non-significant due to different ULN in ALT for males and females.

Table 4

Liver-related biomarkers/ratios by gender, age, and phenotype in CFLD patients

Description		*n*	APRi[a]	FIB-4[b]	Age[c], years	Platelets, ×10^9/L	AST, IU/L	ALT, IU/L
All CFLD Patients	All	497	1.15 (0.66, 2.05)	1.16 (0.61, 2.04)	16.1 (12.3, 21.2)	101 (63, 161)	45 (31, 67)	41 (28, 68)
	Male	310	1.23 (0.68, 2.18)	1.25 (0.63, 2.22)	16.0 (12.1, 20.5)	98 (61, 157)	47 (32, 69)	44 (30, 72)
	Female	187	1.05 (0.63, 1.73)	1.06 (0.61, 1.92)	16.9 (12.4, 21.8)	110 (67, 166)	41 (30, 62)	38 (26, 56)
	p value[d]		**0.038**	0.308	0.155	0.295	**0.029**	**0.011**[e]
Age < 18 years	All	302	1.25 (0.74, 2.10)	1.01 (0.49, 1.74)	13.1 (10.5, 15.6)	103 (69, 160)	48 (37, 73)	43 (31, 71)
	Male	197	1.34 (0.74, 2.23)	1.08 (0.54, 1.78)	13.2 (10.4, 15.7)	103 (65, 153)	49 (38, 78)	46 (30, 73)
	Female	105	1.16 (0.75, 1.77)	0.80 (0.43, 1.56)	12.9 (10.8, 15.6)	102 (72, 174)	47 (36, 66)	41 (32, 64)
	p value[d]		0.102	0.108	0.774	0.416	0.169	0.353
Age ≥ 18 years	All	195	0.99 (0.58,1.99)	1.56, (0.83, 2.78)	22.5 (19.9, 26.0)	100 (58, 164)	36 (27, 57)	39 (24. 62)
	Male	113	1.05 (0.58, 2.04)	1.62 (0.79, 2.91)	22.4 (19.6, 25.8)	90 (56, 167)	39 (27, 58)	43 (29, 68)
	Female	82	0.88 (0.55, 1.56)	1.40 (0.85, 2.37)	22.7 (20.2, 26.3)	113 (63, 160)	34 (26, 53)	36 (21, 51)
	p value[d]		0.329	0.658	0.489	0.523	0.213	**0.010**[e]
***p* value by age[f]**	All		**0.004**	**<0.0001**	not applicable	0.409	**<0.0001**	**0.007**[e]
	Male		**0.031**	**0.0001**	not applicable	0.411	**<0.0001**	0.264
	Female		0.078	**<0.0001**	not applicable	0.632	**0.0002**	**0.006**[e]
Known varices	No varices	139	0.90 (0.48, 1.48)	0.70 (0.41, 1.26)	15.3 (11.2, 19.3)	145 (87, 198)	45 (31, 65)	46 (31, 70)
	Varices	335	1.27 (0.72, 2.29)	1.47 (0.81, 2.47)	17.6 (13.7, 22.2)	88 (56, 142)	44 (31, 65)	41 (27, 66)
	p value[d]		**<0.0001**	**<0.0001**	**0.001**	**<0.0001**	0.691	0.100
Liver transplant	No transplant	422	1.06 (0.62, 1.99)	1.05 (0.57, 1.87)	17.0 (12.7, 21.4)	113 (70, 171)	45 (31, 65)	42 (29, 69)
	Transplant	68	1.59 (1.11, 2.69)	1.96 (1.18, 3.25)	13.3 (10.2, 17.1)	78 (54, 99)	45 (32, 72)	38 (27, 59)
	p value[d]		**0.0001**	**<0.0001**	**0.0005**	**<0.0001**	0.549	0.349

p values (<0.05 shown in bold) generated using Wilcoxon rank sum test (for medians).

Data presented as median (25th and 75th percentiles).

[a] APRi = (AST [IU/L]/ULN AST*100)/platelet count [10^9/L]. ULN for AST was 40 IU/L.

[b] FIB-4 = (Age [years] * AST [IU/L])/(platelet count [10^9L] * square root ALT [IU/L]).

[c] Age, in years = age at blood test.

[d] *p* value comparing males vs. females

[e] The apparent difference between genders is clinically non-significant due to different upper limit of number (ULN) in ALT for males and females.

[f] *p* value by age comparing patients with blood tests performed < 18 vs. ≥ 18 years of age.