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Heterogeneous Liver on Research Ultrasound Identifies Children with Cystic Fibrosis at High Risk of Advanced Liver Disease: Interim Results of a Prospective Observational Case-Controlled Study

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Abstract

Objective: To assess if a heterogeneous pattern on research liver ultrasound can identify children at risk for advanced cystic fibrosis liver disease (aCFLD).

Study design: Planned 4-year interim analysis of a 9-year multicenter case-controlled cohort study (Prospective Study of Ultrasound to Predict Hepatic Cirrhosis in CF). Children with pancreatic insufficient CF aged 3–12 years without known cirrhosis, *Burkholderia* species infection or short bowel syndrome underwent screening US. Participants with HTG US pattern were matched (by age, *Pseudomonas* infection status and center) 1:2 with participants with normal pattern. Clinical status and laboratory data were obtained annually and US biannually. The primary endpoint was the development of a nodular US pattern, a surrogate for aCFLD.

Results: 722 participants underwent screening US, of which 65 were HTG and 592 NL. The final cohort included 55 HTG and 116 NL participants. All participants with at least one follow-up

US were included. There were no differences in age or sex between groups at entry. ALT (mean ± SD) (42±22 U/L vs 32±19, p=0.0033), GGTP (36±34 U/L vs 15±8, $P < .001$) and APRI (0.7±0.5 vs 0.4±0.2, p<0.0001) were higher in HTG compared with NL. HTG participants had a 9.1-fold increased incidence (CI:2.7, 30.8, p=0.0004) of NOD pattern versus NL (23% in HTG vs 2.6% in NL).

Conclusions: Research liver US can identify children with CF at increased risk for developing aCFLD.

Keywords

cystic fibrosis liver disease; cirrhosis

Despite the recognition of liver involvement in the pathologic process of cystic fibrosis (CF), the identification and classification of CF liver disease (CFLD) remains problematic[1,2]. This has been primarily due to the lack of reliable sensitive and specific diagnostic markers to predict liver involvement in CF before cirrhosis with portal hypertension is present. Advanced CFLD (aCFLD) manifest as portal hypertension with or without cirrhosis occurs in about 7% of individuals with CF[3,4]; the median age at diagnosis is 10 years[5]. Risk factors for the development of aCFLD include male sex and Class 1–3 cystic fibrosis transmembrane regulator (CFTR) mutations[6,7]. The heterozygous state for the z allele of alpha 1 antitrypsin is associated with a 7-fold increased risk for the development of aCFLD[8]. However, this was only present in 9% of participants with aCFLD[8]. A high gamma glutamyl transpeptidase (GGTP) activity early in life is also associated with an increased risk for the development of aCFLD[9,10]. As new therapies are developed for CF and other liver diseases, that may be relevant to CFLD, there is a need for prospective, large scale studies to attempt to identify sensitive and specific biomarkers that can distinguish individuals with CF at increased risk for the development of aCFLD.

A heterogeneous echogenic pattern of the liver on abdominal ultrasound has been suggested, in a previous single center study, to identify individuals at risk for aCFLD (defined as a nodular liver on US) with or without portal hypertension) and could potentially be used as a predictive biomarker for the risk of aCFLD[11]. CF participants with a HTG pattern on US had a 5.2-fold increased incidence of a NOD US and a 6.1-fold increased incidence of portal hypertension compared with participants with a normal echogenic pattern on US[11]. Based on these data, the Cystic Fibrosis Liver Disease Network (CFLD-NET) undertook a study to determine if US is an effective tool to screen for the risk of the development of aCFLD. Herein, we report the results of the planned 4-year interim analysis of the Prospective Study of Ultrasound to Predict Hepatic Cirrhosis in CF (PUSH) (ClinicalTrials.gov: NCT 01144507).

METHODS

CFLD-NET is a North American multicenter group which includes 11 clinical sites and a data coordinating center. CFLD-NET initiated a prospective multicenter case-controlled cohort study to investigate the utility of abdominal US to identify young children with CF at risk for the development of aCFLD (PUSH study). The protocol was reviewed and approved

by the Institutional Review Boards at all centers. This study was optionally registered at ClinicalTrials.gov (NCT 01144507). All authors had access to the study data and reviewed and approved the final manuscript. Study participants were recruited between January 2010 and February 2014. All guardians provided informed consent and appropriate assent was obtained. Children 3–12 years of age were eligible for the study based on the following inclusion criteria: (1) diagnosis of CF determined by a sweat chloride of >60 mEq/L or 2 disease-causing CFTR genetic mutations with evidence of end organ involvement; (2) enrollment in either the Cystic Fibrosis Foundation (CFF) or Toronto CF registry; and (3) diagnosis of pancreatic insufficiency. Exclusion criteria were known cirrhosis or portal hypertension (ie, splenomegaly, ascites), prior identification of *Burkholderia* species on respiratory culture, or short bowel syndrome.

Data collected included demographics, growth measures, physical findings, CFTR genotype, routine clinically indicated laboratory values and research US findings. The CFF and Toronto CF registries were used for historical and clinical data, including medical insurance, weight and height, symptoms at diagnosis, history of malnutrition, infection, lung function, complications, and medications. All data was integrated in a centralized database at the data coordinating center.

Research US was performed with both gray-scale and Doppler imaging at each site. Grading followed the system of Williams et al.[12] Liver echogenicity and contours were assessed to classify a patient into 1 of 4 US patterns. NL denoted normal hepatic echogenicity. HTG denoted increased echogenicity that was diffusely patchy or limited to periportal regions. Homogeneous denoted diffusely increased hepatic parenchymal echogenicity relative to renal echogenicity, absent or poor definition of portal venous and hepatic structures, and posterior beam attenuation with absent or incomplete diaphragm visualization. NOD pattern denoted a heterogeneous echotexture of the liver parenchyma and obvious nodularity of the liver contour (Figure 1; available at www.jpeds.com). There was a single study radiologist at each site for the duration of the study. Study radiologists have an average of 20 years' experience. The study radiologist from each site completed web-based training for the grading of the US studies. A training set of representative images from each grade was developed by the lead study radiologist Validation of consistency (kappa statistics > 0.7) in the readings was assessed with the training set prior to study initiation. The lead study radiologist also reviewed the first 5 US studies from every site for quality to ensure uniform quality and validated degree of concordance before study continuation. Sonographer training included the same training set used for the radiologists with a written guide documenting the required images for the study.

At study entry, each participant completed a quality of life survey and underwent a standardized research US to include a detailed examination of the liver and spleen to assess for the presence or absence of liver disease and a gray-scale survey examination of the entire abdomen to assess for ancillary findings. Each US was independently graded by 4 study radiologists: the local study radiologist from each center participating in the PUSH study and 3 additional (2 primary and 1 back up) study radiologists randomly assigned from the different participating study sites in regular rotation. All radiologists were blinded to the results of the other interpretations, prior US studies and clinical data. The consensus grade

was assigned by majority of the local and 2 primary radiologists. In the absence of consensus among the 3 primary readers, the back-up radiologist read was used to establish consensus (n=21, 2.9% of 723 screened participants). If 4 different grades were submitted, the patient was excluded from the study (n=1, 0.4%).

HTG participants were matched with 2 NL participants by age (±2 years), center and *Pseudomonas* infection status. The HTG and matched NL participants were enrolled in longitudinal follow up planned for up to 9 years. In longitudinal follow up, participants underwent annual evaluations with physical examination, laboratory data collection, biospecimen collection and quality of life surveys. Follow up US was, or will be performed at year 2, 4, 6 and 8 (±4 months).

The primary endpoint for the study is the development of a NOD US pattern by year 6 of follow up based on the consensus of 2 of the 3 primary study radiologist readers. Given the potential impact of early evidence of the utility of US to identify children at risk for aCFLD, a planned interim analysis was included in the study design. For this analysis, the primary endpoint is the development of NOD by year 4 of follow up based on a more stringent criterion: the consensus of at least 3 of 4 study radiologist readings. The last year 4 US was completed on Jan 1, 2018 with the last consensus US grade completed on Apr 3, 2018. Laboratory and physical data from the visit closest to the year 4 US is referred to as data at the most recent US.

Statistical Analyses

Data presented are mean ± standard deviation for counts and percentages. For comparing the proportion of participants developing NOD by year 4 between groups, we used a Fisher exact test with type I error 0.025 to adjust for potential multiple testing with the interim analysis. We investigated additional predictors that can potentially improve the prediction of risk for development of NOD compared with the use of US alone, including age at enrollment, sex, ethnicity, history meconium ileus, early *Pseudomonas* infection, newborn screening diagnosis, previous ursodeoxycholic acid use, GGTP, AST, ALT, albumin, platelet count, age-adjusted spleen size z-score[13], height-adjusted portal vein diameter z-score[14], AST to platelet ratio index (APRI), FIB-4, height z-score, and weight z-score (CDC, https://www.cdc.gov/nccdphp/dnpao/growthcharts/resources/sas.htm). We first created a logistic regression model for development of NOD at year 4 with baseline US as the single covariate. We then added each of the above risk factors one at a time into this logistic regression model. We refer to this analysis as univariate analysis. This analysis allows us to explore whether any single additional predictors can improve the prediction of using US information alone. Given the small number of events (only 16 participants developed NOD) observed, this dataset is not suitable for developing/exploring better prediction models. We did not conduct multivariate analysis. We calculated summary statistics for baseline demographic, laboratory, physical examination, and medical history features; as well as summary statistics for lab and physical examination features at the of year 4 US. For testing difference between groups, Wilcoxon or t-test were used for continuous variables, and Fisher exact test or Chi-square test were used for categorical variables.

RESULTS

A total of 774 participants were enrolled. 722 participants had a consensus grade at screening, of whom 65 were HTG and 592 NL. Participants with baseline HMG or NOD are not included in this analysis. The consort diagram is presented in Figure 2 (available at www.jpeds.com). Baseline data from all participants was previously reported[15]. This study focuses on the ability, in isolation, and in association with other risk factors, of US to identify participants at risk of aCFLD defined as a NOD pattern. All participants with at least one follow-up US were included. The final cohort for this analysis included 55 HTG and 116 NL. Participants missing their year 4 US had their year 2 US utilized (n=2 for HTG and 6 for NL). The demographic and laboratory information for these participants is shown in Table 1. Participants were well matched for age and *Pseudomonas* infection status. There were more males in the HTG group. There were significant differences in GGTP, AST, ALT, platelets, AST to Platelet Ration Index (APRI) and FIB-4 at baseline between HTG and NL, with substantial overlap of the range of values.

Over the first four years of follow up, there was a significant difference between HTG and NL for the development of NOD; 23% (13 of 55) of participants with a baseline HTG pattern developed a NOD pattern within 4 years compared with only 2.6% (3 of 116) with a baseline NL pattern (Table II). This difference translates into a relative risk of 9.1 (95% confidence interval (CI): 2.7, 30.8) for the subsequent development of a NOD US pattern for participants with a research-based HTG US pattern at study entry. We then investigated differences in laboratory and clinical data at baseline between participants who developed NOD and those that did not (Table 3). HTG participants that developed NOD had significantly higher GGTP, AST, weight for age z score (WAZ), and lower proportion of meconium ileus history at baseline. This group also had higher APRI (p=0.054) at baseline, although not statistically significant. Because there were only 3 participants with NL US who developed NOD, we did not perform a formal comparison of NL to NL who developed NOD.

The laboratory and clinical data at the last US visit (values from the visit closest to the year 4 US) for each group are shown in Table 4. HTG participants who developed NOD pattern had higher GGTP, AST, ALT, APRI, FIB-4, spleen size z-scores, and lower platelet count than HTG participants without NOD. There was a more significant difference in these values between the participants with HTG with NOD and those with HTG without NOD at their last US visit compared with baseline differences.

Univariate logistic regression (Table 5; available at www.jpeds.com) showed that baseline AST, albumin, APRI, and WAZ are associated with development of NOD independent of US pattern at screening. The ROC curve for prediction of NOD demonstrated an area under the curve (AUC) of 0.77 in participants with HTG baseline US (versus NL). Thus, the sensitivity and specificity of gray-scale US in the PUSH longitudinal follow-up population is 81.3% and 72.9%. Adding the most significant additional predictor, APRI into the model improved the AUC of ROC curve from 0.77 to 0.83 (Figure 3; available at www.jpeds.com).

Discussion

With advances in CFTR directed therapies and other potential liver specific therapies in development, it is important to identify individuals with CF at high risk for CFLD. To that end, we have presented the interim results from a large multicenter trial investigating the utility of a research-based US to identify individuals with CF at high risk for the development of aCFLD. the goal was to identify factors that would predict the subsequent development of aCFLD. We chose US due to the availability across the clinical centers. Because aCFLD occurs predominantly in children, risk stratification for liver disease would ideally utilize minimally invasive, readily available and affordable techniques. We have demonstrated that a HTG US pattern, using a standardized research US with consensus from 4 radiologists with specialized training, identifies participants at high risk for the development of a NOD liver pattern associated with aCFLD. The isolated finding of a HTG US pattern is associated with a 9-fold increased risk for the development of a NOD US pattern consistent with aCFLD with a ROC AUC of 0.77. This is consistent with a single center study which reported a 5.6-fold increased risk for the subsequent development of aCFLD over 10 years in children and adolescents with CF and NOD[11]. In that study, the radiologist was not blinded to clinical data or prior US data. They found a higher frequency of HTG (14.2%: 15/106) than in our study (8.7%, 63/723) with both studies using the same definition. We speculate that this difference may reflect the role of training and consensus readings, underscoring the importance of a standardized training protocol like that used in this study.

Analysis of the HTG US data suggest that in addition to US pattern there may be other laboratory findings that identify individuals with CF at increased risk for developing aCFLD. In participants with similar HTG US finding at baseline, there were significant differences in baseline AST and GGTP between the group that developed NOD and the group that did not. However, the clinical utility of using AST and GGTP in predicting the development of NOD pattern in the setting of a HTG pattern is limited due to significant overlap of the values. With multivariate modeling, we found that the addition of APRI at the time of the identification of HTG US pattern into the model did slightly improve the AUC for predicting subsequent development of aCFLD. Our data are also consistent with reports of higher GGTP in individuals at risk for or with aCFLD[9,10], and higher APRI in those individuals with aCFLD[16,17]. The findings of this interim analysis do not identify specific clinical risk factors that predict the development of NOD pattern.

With respect to potential pulmonary contributors to aCFLD, we did not observe a relationship between early *Pseudomonas* infection and the subsequent development of NOD. Our findings from a previous study reported that early (before 2 years of age) *Pseudomonas* infection at baseline was protective for the finding of any US abnormality (HTG, HMG or NOD)[15].

Additionally, as shown in Table 4, the participants with HTG that developed a NOD pattern had lower platelets, a larger spleen and higher APRI and FIB-4 at the time of the last US, compared with those with a HTG US at baseline that did not develop NOD, consistent with more advanced liver disease and portal hypertension. This may be related to cirrhosis and

portal hypertension or the more recently recognized nodular regenerative hyperplasia that has also been reported in aCFLD[18,19]. This supports the idea that the finding of NOD is associated with aCFLD and is similar to a prior report of an association between lower platelet count and aCFLD[20].

These research data may not immediately translate to the interpretation of routine ultrasounds or predict the risk of progression to aCFLD in the clinical realm. We point out that we used consensus reads in this study by radiologists who underwent specific training and who were blinded to clinical data.

Although this study has identified a subset of children with CF who are at high risk for aCFLD, 75% of those with a HTG US had not developed aCFLD by 4 years. Thus, further refinement with the addition of biomarkers, elastography or other standardized imaging findings will likely be needed to optimize the identification of children at a high risk for aCFLD.

Our study has several limitations. First, no histological correlation was available for the imaging patterns that are used in the grading system as liver biopsy was not part of this study. However, a NOD pattern is recognized as a finding of advanced liver disease, such as cirrhosis, on US. Second, our follow up is shorter than that reported by Lenaerts et al, who found a 5.6-fold increased risk for the subsequent development of aCFLD in children and adolescents with a NOD pattern over a longer 10-year period[11]. Ongoing clinical follow-up of our large study cohort is planned to further define the utility of screening abdominal US and the significance of the spectrum of US findings in young children with CF. Third, we did not incorporate other imaging technologies, such as elastography, which measures liver stiffness and has been shown to correlate with advanced fibrosis in pediatric liver diseases including CFLD[21,22]. We recognize that since the beginning of this study elastography has been demonstrated to have utility as a marker of advanced liver disease including CF. Elastography was not included as an endpoint in this study as it was not readily available for pediatric use in 2010. Studies in CF have shown that identification of advanced liver disease by physical examination or US does correlate with elastography findings of F3 or F4[23,24]. Thus, our focus was on the use of conventional US, which is widely available, to optimize the identification of children at a risk for aCFLD.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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List of abbreviations:

APRI	AST to Platelet Ratio Index

CF	cystic fibrosis
CFLD	cystic fibrosis liver disease
aCFLD	advanced cystic fibrosis liver disease
CFTR	cystic fibrosis transmembrane regulator
GGTP	gamma glutamyl transpeptidase
CFLD NET	Cystic Fibrosis Liver Disease Network
CFF	Cystic Fibrosis Foundation

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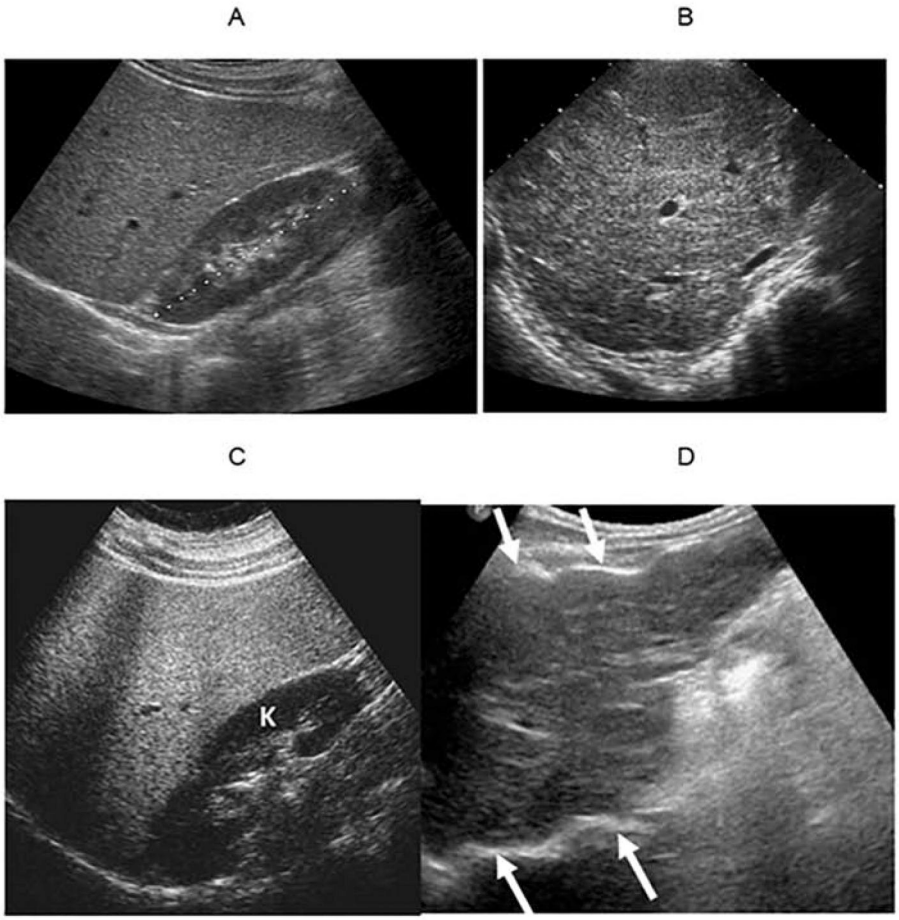

Figure 1 (online only):
Liver Ultrasound (US) Pattern Grading
A: Normal US. Normal parenchyma is slightly more echogenic than the kidney (dashed line). B: Heterogeneous US showing patchy increased echogenicity denoted as bright areas within the liver, C: Homogeneous US. Liver echogenicity is diffusely increased relative to the echogenicity of the right kidney (K). D: Nodular US characterized by patchy areas of increased echogenicity and nodular liver contours (arrows).

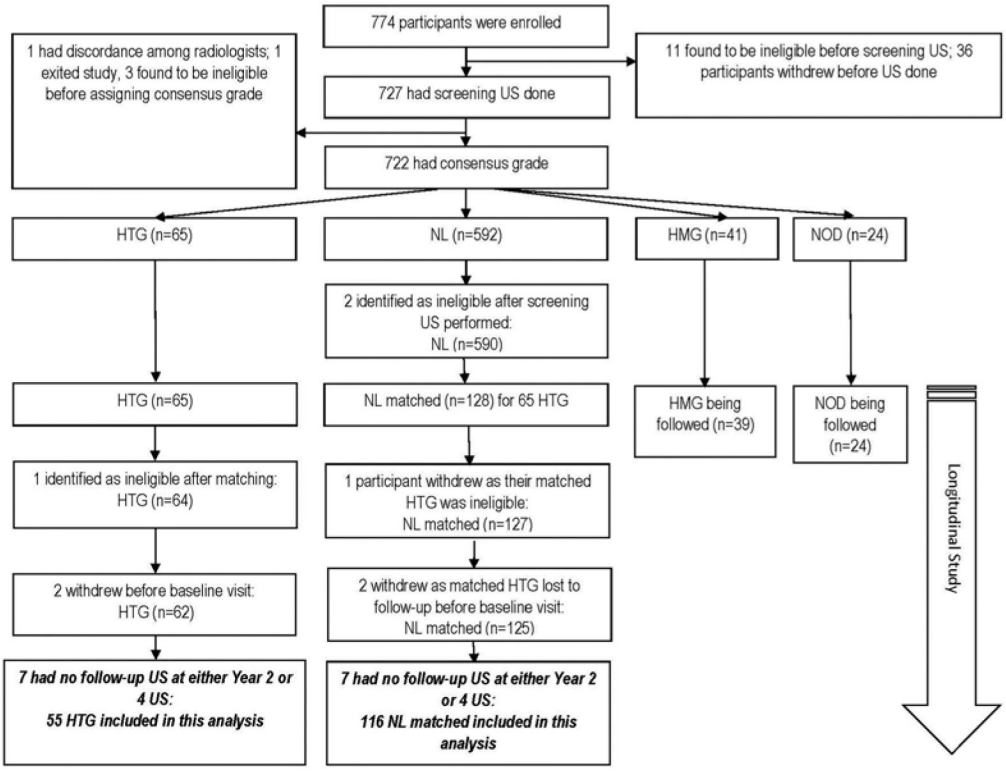

Figure 2 (online only):
Consort Diagram
US: Research ultrasound, NL: Normal liver US pattern, HTG: Heterogeneous liver US pattern, HMG: Homogeneous liver US pattern, NOD: nodular liver US pattern

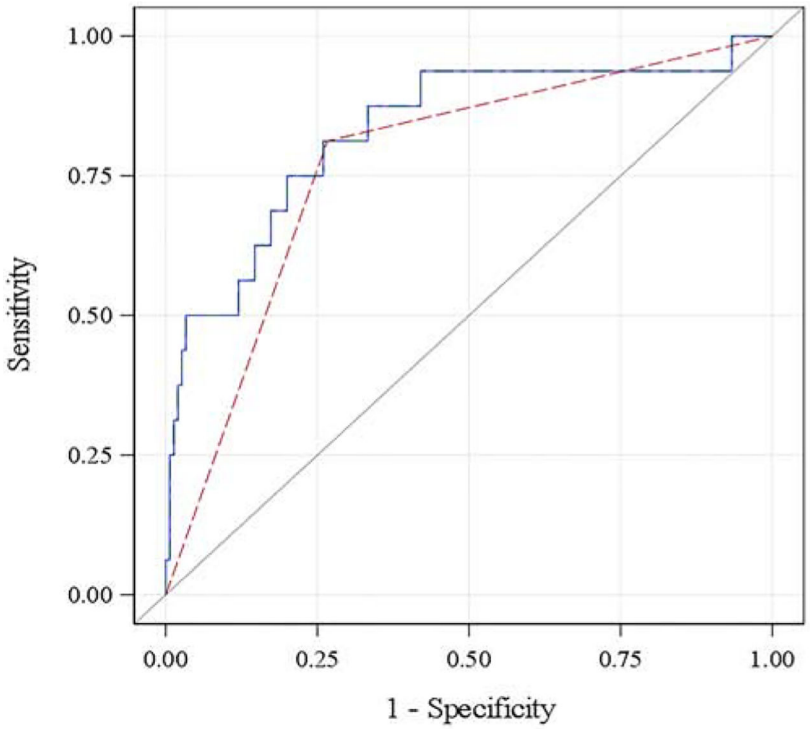

Figure 3 (online only):

Receiver operating characteristics (ROC) curves

ROC curves for prediction of NOD pattern (HTG vs NL) using baseline US grade alone (dashed line, area under the curve=0.77) and for final model (Baseline US grade (HTG vs NL) and APRI-solid line, area under the curve=0.83)

Table 1:

Baseline characteristics of participants by US consensus grade at screening

	HTG N=55	NL N=116	P value
Demographics / Matching Features			
Age (years), mean (SD)	8.7 (3.2) 9.2(3.1, 13.1) N= 55	8.4 (3.1) 8.9(3, 13.4) N= 116	0.51[a]
Pseudomonas positive at enrollment, n (%)	15 (24.19%) N = 62	28 (22.76%) N = 123	0.83[a]
Male Gender, n (%)	35 (63.64%) N= 55	58 (50.00%) N= 116	0.095[b]
Hispanic, n (%)	0 (0.00%) N= 55	7 (6.03%) N= 116	0.098[c]
Lab and physical exam			
GGTP (U/L), mean (SD) Median (min, max)	35.9 (34.3) 23(7, 204) N= 51	15.2 (7.7) 13(4,46) N= 106	**<.0001**[d]
AST (U/L), mean (SD) Median (min, max)	45.3 (30) 39(22, 236) N= 54	34.1 (12.9) 31(16, 108) N= 114	**<.0001**[d]
ALT (U/L), mean (SD) Median (min, max)	42 (22.4) 41(10,97) N= 51	31.6 (18.8) 27(6, 154) N= 110	**0.0033**[d]
Albumin (g/dL), mean (SD) Median (min, max)	4.3 (0.4) 4.3(3.2, 5.3) N= 50	4.2 (0.4) 4.3(3.1,5) N= 106	0.64[d]
Platelet (10^3/mm^3), mean (SD) Median (min, max)	300.5 (77.5) 299(108,527) N= 55	332.9 (71.7) 322(203, 532) N= 114	**0.008**[e]
Spleen size Z-score, mean (SD) Median (min, max)	0.3 (1.7) 0.1(−4.2, 4.4) N= 55	0 (1.2) 0(−2.9, 4.1) N= 116	0.27[e]
Portal vein diameter Z-score, mean (SD) Median (min, max)	0.4 (1.2) 0.5(−1.9, 3.3) N= 54	0.4 (1.1) 0.2(−1.8, 3.6) N= 111	0.94[e]
APRI, mean (SD) Median (min, max)	0.7 (0.5) 0.6(0.2, 3.7) N= 54	0.4 (0.2) 0.4(0.2, 1.6) N= 113	**<.0001**[d]
FIB-4, mean (SD) Median (min, max)	0.2 (0.2) 0.2(0.1, 1) N= 51	0.2 (0.1) 0.1(0,0.4) N= 108	**0.006**[d]
Height-age Z-score, mean (SD) Median (min, max)	−0.1 (1) −0.2 (−1.9, 1.8) N= 54	−0.2(1) −0.2(−2.8,2.2) N= 112	0.48[e]
Weight-age Z-score, mean (SD) Median (min, max)	−0.1 (0.9) −0.1 (−2.2, 1.8) N= 54	−0.2 (0.9) −0.1 (−3.7, 2) N= 112	0.61[e]
Medical History			
Meconium ileus present, n (%)	11 (20.00%) N= 55	24 (20.69%) N= 116	0.92[b]
Newborn screen diagnosis, n (%)	14 (25.45%) N= 55	28 (24.14%) N= 116	0.85[b]
Early *Pseudomonas* (≤ 2 years), n (%)	22 (46.81%) N= 47	52 (54.17%) N= 96	0.41[b]

[a] Wilcoxon test

[b] Chi-square test

[c] Fisher's exact test

[d] Two sample t-test based log-transformed scale

[e] Two sample t-test based on original scale

Table 2:

Relative risk of development of nodular US liver pattern in children with CF by baseline US grade

US screen grade	N	NOD US at last follow up	Non-NOD US at last follow up	Relative Risk of NOD (95% CI)	p-value
HTG	55	13 (23.6%)	42 (76.4%)	9.1 (2.7, 30.8)	0.0004
NL	116	3 (2.6%)	113 (97.4%)		

Table 3.

Comparison of participant characteristics at baseline visit grouped by final US consensus grade

	HTG without NOD development N=42	HTG with NOD development N=13	P Value[a]	NL without NOD development N=113	NL with NOD development N=3[g]
Demographics					
Age (years), mean (SD)	9.1 (3.2) 10.2 (3.1, 13.1) N= 42	7.2 (2.8) 6.7 (3.4, 12.5) N= 13	0.064[b]	8.4 (3.1) 9.1 (3.0, 13.4) N= 113	7.7 (2.5) 7.2 (5.5, 10.3) N= 3
Pseudomonas at baseline n (%)	11 (26.19%) N= 42	3 (23.08%) N= 13	1.00[d]	27 (23.89%) N= 113	1 (33.33%) N = 3
Male Gender, n (%)	25 (59.52%) N= 42	10 (76.92%) N= 13	0.33[d]	57 (50.44%) N= 113	1 (33.33%) N= 3
Hispanic, n (%)	0 (0.0%) N= 42	0 (0.0%) N= 13	-	7 (6.19%) N= 113	0 (0.0%) N= 3
Lab and Physical exam					
GGTP (U/L), mean (SD) Median (min, max)	29.1 (20.8) 21.5 (7,82) N= 38	55.6 (54.9) 25 (11, 204) N= 13	**0.044**[e]	15.1 (7.7) 13 (4,46) N= 104	16.5 (0.7) 16.5 (16, 17) N= 2
AST (U/L), mean (SD) Median (min, max)	43.3 (33.2) 37 (22, 236) N= 41	51.8 (15.5) 54 (30, 74) N= 13	**0.046**[e]	34.1 (13.0) 31 (16, 108) N= 111	34 (3.6) 33 (31, 38) N= 3
ALT (U/L), mean (SD) Median (min, max)	38.8 (20.7) 39 (10, 95) N= 39	52.2 (25.5) 49.5 (13,97) N= 12	0.11[e]	31.7 (19.0) 27 (6, 154) N= 107	28 (4.6) 27 (24, 33) N= 3
Albumin (g/dL), mean (SD) Median (min, max)	4.2 (0.4) 4.2 (3.2, 5.1) N= 39	4.5 (0.3) 4.4 (4, 5.3) N= 11	0.062[e]	4.2 (0.4) 4.3 (3.1,5.0) N= 103	4.4 (0.3) 4.6 (3.9, 4.7) N= 3
Platelet (10^3/mm^3), mean (SD) Median (min, max)	303.2 (73.7) 299.5 (108, 456) N= 42	291.8 (91.5) 292 (159, 527) N= 13	0.65[f]	333.2 (69.3) 322 (203,521) N= 111	338.3 (167.8) 246 (237, 532) N= 3
Spleen size Z-score, mean (SD) Median (min, max)	0.25 (1.81) 0.10 (−4.19, 4.40) N= 42	0.41 (1.16) 0.20 (−1.29, 3.04) N= 13	0.76[f]	0.02 (1.23) 0 (−2.94, 4.13) N= 113	−0.35 (0.34) −0.38 (−0.68, 0) N= 3
Portal vein diameter Z-score, mean (SD) Median (min, max)	0.4 (1.3) 0.6 (−1.9,3.3) N= 41	0.3 (1.2) 0.1 (−1.3,2.0) N= 13	0.83[f]	0.4 (1.1) 0.2 (−1.8,3.6) N= 107	0.0 (1.1) −0.3 (−0.9, 1.3) N= 3
APRI, mean (SD) Median (min, max)	0.6 (0.5) 0.5 (0.2,3.7) N= 41	0.8 (0.3) 0.8 (0.3, 1.2) N= 13	0.054[e]	0.4 (0.2) 0.4 (0.2, 1.6) N= 110	0.5 (0.2) 0.5 (0.2, 0.6) N= 3
FIB-4, mean (SD) Median (min, max)	0.2 (0.2) 0.2 (0.1, 1.0) N= 39	0.2 (0.1) 0.2 (0.1,0.4) N= 12	0.80[e]	0.2 (0.1) 0.1 (0.0, 0.4) N= 105	0.2 (0.1) 0.2 (0.1,0.3) N= 3
Height-age Z-score, mean (SD) Median (min, max)	−0.2 (1.0) −0.3 (−2.0, −1.8) N= 41	−0.1 (0.9) −0.1 (−1.8, 1.1) N= 13	0.81[f]	−0.3 (1.0) −0.3 (−3.3, 2.2) N= 108	0.0 (0.2) −0.0 (−0.1. 0.2) N= 3
Weight-age Z-score, mean (SD) Median (min, max)	−0.3 (0.9) −0.3 (−2.0, 1.8) N= 41	0.3(1.0) 0.6 (−2.2, 1.4) N= 13	**0.041**[f]	−0.2 (0.9) −0.1 (−3.7, 2.0) N= 108	0.1 (0.6) 0.1 (−0.5, 0.6) N= 3
Medical History					
NB screen diagnosis, n (%)	11 (26.19%) N= 42	3 (23.08%) N= 13	1.00[d]	27 (23.89%) N= 113	1 (33.33%) N= 33.33%)
Early Pseudomonas, n (%)	16 (44.44%) N= 36	6 (54.55%) N= 11	0.56[c]	51 (54.26%) N= 94	1 (50.00%) N= 2
UDCA use, n (%)	6 (15.79%) N= 38	3 (27.27%) N= 11	0.40[d]	7 (7.14%) N= 98	0 (0.00%) N= 3
Meconium ileus, n (%)	11 (26.19%) N= 42	0 (0.0%) N= 13	0.050[d]	24 (21.24%) N= 113	0 (0.0%) N= 3

[a] All p-values are comparing HTG with NOD development vs. HTG without NOD development

[b] Kruskal-Wallis test

[c] Chi-square test

[d] Fisher's exact test

[e] T-test based log-transformed scale

[f] T-test based on original scale

[g] Comparison between NL without development of NOD and NL with development of NOD was not performed due to the low number (3) in the NL with development of NOD group.

Table 4:

Laboratory and physical features present at last interim follow up by baseline US consensus grade grouped by final US consensus grade

	HTG without NOD development N=42	HTG with NOD development N=13	P value HTG no NODvs HTG NOD	NL without NOD development N=113	NL with NOD development N=3[a]
Lab and Physical exam					
GGTP (U/L), mean (SD) Median (min, max)	30.7 (26.3) 18 (6, 127) n = 37	83.2 (134.3) 30.5 (15,491) n = 12	**0.020**[b]	16.8 (11.6) 13 (5, 86) n = 103	38 (18.4) 38 (25,51) n = 2
AST (U/L), mean (SD) Vledian (min, max)	37.8 (25.8) 28.5 (10, 146) n = 42	65.6 (56.7) 50.5 (20, 226) n = 12	**0.015**[b]	29.3 (13.5) 26 (8, 87) n = 111	36.3 (3.1) 37 (33, 39) n = 3
ALT (U/L), mean (SD) Vledian (min, max)	39 (26.8) 30 (6, 132) n = 41	60.4 (33.7) 47 (27,126) n = 11	**0.018**[b]	31.1 (17.5) 28 (8, 117) n = 106	24.7 (4.6) 22 (22, 30) n = 3
Platelet (10^3/mm^3), mean (SD) Median (min, max)	276.8 (88.5) 260 (115, 582) n = 41	194.6 (105.5) 191.5 (60, 401) n = 12	**0.0091**[c]	309.7 (68.7) 303 (90, 521) n = 110	265.3 (70.2) 277 (190, 329) n = 3
Spleen size Z-score, mean (SD) Median (min, max)	1 (1.8) 1 (−3.8, 4.6) n = 42	3.6 (3.9) 2.8 (−0.4, 13.5) n = 13	**0.033**[c]	0.4 (1.6) 0.3 (−3.5, 6.9) n = 112	0.2 (0.3) 0.3 (0.1, 0.6) n = 3
Portal vein diameter Z-score, mean (SD) Median (min, max)	1.1 (2.3) 1 (−2.5, 8.6) n = 42	1.3 (1.4) 0.8 (0.6, 4.4) n = 12	0.82[c]	0.5 (1.6) 0.5 (−2.2, 5.1) n = 111	0.5 (1.6) 0.8 (−1.3,1.9) n = 3
APRI, mean (SD) Median (min, max)	0.6 (0.5) 0.5 (0.1,2.9) n = 41	1.7 (1.4) 1.3 (0.3, 4.9) n = 12	**0.0002**[b]	0.4 (0.2) 0.4 (0.1,1.1) n = 109	0.6 (0.2) 0.5 (0.4, 0.8) n = 3
FIB-4, mean (SD) Median (min, max)	0.3 (0.2) 0.3 (0.1, 1.3) n = 40	0.6 (0.4) 0.6 (0.2, 1.2) n = 11	**0.0052**[b]	0.2 (0.1) 0.2 (0.1,0.8) n = 104	0.4 (0.1) 0.4 (0.2, 0.4) n = 3
Height-age Z-score, mean (SD) Median (min, max)	−0.1 (1) −0.2 (−2.2, 2.3) n = 42	0.1 (1.1) 0.4 (−1.6, 2.1) n = 13	0.52[c]	−0.2 (1.3) −0.2 (−6.8, 2.8) n = 112	−0.1 (0.5) 0.2 (−0.6, 0.4) n = 3
Weight-age Z-score, mean (SD) Median (min, max)	−0.2 (1) −0.2 (−2.7, 1.8) n = 42	0.2 (0.9) 0.5 (1.6, 1.4) n = 13	0.20[c]	−0.1 (0.9) 0.1 (−3.1, 1.8) n = 112	−0.2 (0.8) −0.4 (−0.8, 0.7) n = 3

[a] Comparison between NL without development of NOD and NL with development of NOD was not performed due to the low number (3) in the NL with development of NOD group

[b] T-test based log-transformed scale

[c] T-test based on original scale

Table 5.

Univariate logistic regression analysis (all models adjusted for US consensus grade at screening)

Variable	Odds Ratio Estimates	P-value
Age	0.845(0.708, 1.009)	0.063
Gender (female vs. male)	0.658(0.205,2.115)	0.48
Ethnicity (Non-Hispanic vs. Hispanic)	--	0.98
Meconium Ileus (Yes vs. No)	--	0.95
Early *Pseudomonas* (Yes vs. No)	1.347 (0.396, 4.582)	0.63
Newborn Screen Diagnosis (Yes vs. No)	0.986 (0.279, 3.487)	0.98
Previous UDCA use (Yes vs. No)	1.617(0.365,7.159)	0.53
GGTP (U/L)	1.021 (0.996, 1.047)	0.093
AST (UL)	1.044(1.009, 1.079)	0.012
ALT (U/L)	1.017(0.995, 1.041)	0.13
Albumin (g/dL)	5.401 (1.013,28.805)	0.048
Platelet (10^3/mm^3)	0.999(0.991, 1.006)	0.72
APRI	17.403(2.278, 132.957)	0.006
FIB-4	1.262 (0.006, 252.397)	0.93
Spleen Size Z-Score	1.016(0.716, 1.442)	0.93
Portal Vein Diameter Z-Score	0.905(0.572, 1.432)	0.67
Height-age Z-score	1.146(0.646,2.035)	0.64
Weight-age Z-score	2.022 (1.021, 4.004)	0.044