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Risk factors for more rapid progression of severe liver fibrosis in children with cystic fibrosis-related liver disease: A multi-center study validated by liver biopsy

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Abstract

Background and Aims: Early identification of risk factors for the development of severe fibrosis in children with cystic fibrosis-related liver disease (CFLD) is crucial as promising therapies emerge.

Methods: This multi-center cohort study of children with a priori defined CFLD from 1999 to 2016, was designed to evaluate the clinical utility of CF-specific characteristics and liver biomarkers assessed years prior to liver biopsy-proven CFLD to predict risk of developing severe fibrosis (F3-4) over time. Fibrosis was staged by Metavir classification.

Results: The overall study cohort of 42 patients (F0-2 ($n=22$) and F3-4 ($n=20$)) was 57% male ($n=24$) with median age of 7.6 years at baseline visit versus 10.3 years at biopsy. Median FEV_1% predicted was lower in F3-4 participants at baseline versus F0-2 (59% vs. 85%; $p=.002$), while baseline FIB-4, APRI and GGT were higher in F3-4. Median splits for FIB-4 (≥.13), APRI (≥.36), GPR (≥.09), GGT (≥25.5), and FEV_1% (<64%) were associated with more rapid progression to F3-4 ($p<.01$ for all). Using a combination of change/year in FIB-4, APRI, and GPR to predict F3-4, the AUROC was .81 (95% CI, .66, .96; $p<.0001$). For up to 5.8 years prior, thresholds for GPR were met 6.5-fold more rapidly, and those for APRI and FIB-4 were met 2.5-fold more rapidly, in those who progressed to F3-4 than those that did not.

Conclusions: This study suggests mild–moderate pulmonary dysfunction and higher liver biomarker indices at baseline may be associated with faster progression of CFLD.

KEYWORDS
biomarkers, cystic fibrosis, liver disease, severe liver fibrosis, time to progression

Abbreviations: APRI, aspartate aminotransferase-to-platelet ratio index; ATS, American Thoracic Society; CFLD, cystic fibrosis-related liver disease; CFRD, CF-related diabetes mellitus; FIB-4, fibrosis-4 index; GGT, gamma glutamyl transferase; GPR, GGT-to-platelet ratio; UDCA, ursodeoxycholic acid; ULN, upper limit of normal; US, ultrasound.

1 | INTRODUCTION

Cystic fibrosis-associated liver disease (CFLD) has long plagued clinicians due to non-uniform diagnostic criteria[1–3] and limited therapies to halt or reverse progression to severe CFLD with portal hypertension or cirrhosis. It has been estimated that 5% to 10% of CF participants are diagnosed with liver cirrhosis or portal hypertension[4,5] within the first decade of life[6] and mortality due to CFLD is reported as the primary cause of death in 3.4%,[7] but considered a contributing factor to premature mortality from all causes in many more patients.[8]

Identifying patients with CF who are at higher risk for progression to severe liver disease has been equally challenging. Severe class I-III CFTR mutations, alpha-1-antitrypsin ZZ genotype[9,10] and a heterogeneous echogenic ultrasound pattern represent established or reproducible clinical predictors that have permitted early risk-stratification of participants with CFLD who are more likely to develop severe fibrosis[4,11] and hence decreased survival.[12] However, it is unknown if progression to severe CFLD occurs gradually over time or in discrete rapid cycles. Data regarding timing of disease progression would inform more effective early surveillance for CFLD.[1–3,13,14] While the reference standard has historically been liver biopsy, promising modalities to investigate liver involvement in CF and assess risk for severe fibrosis have included elastography,[15–19] microRNAs,[20,21] gamma glutamyl transferase (GGT), fibrosis-4 index (FIB-4) and aspartate aminotransferase-to-platelet ratio index (APRI).[16,20,22,23,24] GGT-to-platelet ratio (GPR) has been studied in non-CF liver disease[25,26] and its utility as a biomarker of liver disease, portal hypertension and hepatic fibrosis severity has recently been demonstrated in children with CF.[27,28]

This study aims to evaluate the clinical utility of baseline and change in biomarker indices, CF-specific clinical features and specific laboratory parameters over time in paediatric CFLD to predict the risk of developing, and time taken to develop, severe (F3-4) fibrosis. Early identification of the risk of developing significant liver disease and timing of this process is crucial as highly effective modulator therapies and other candidate drugs which may reverse CFLD emerge.

Key points

- Patients with cystic fibrosis-associated liver disease (CFLD) are at high risk of morbidity and mortality due to portal hypertension or cirrhosis.
- Identification of patients with CF who are at higher risk of progression to severe liver fibrosis is challenging, as is the timing of progression.
- Patients with moderate lung dysfunction (FEV1 <64%) develop severe fibrosis faster than patients with normal lung function.
- GGT, FIB-4, APRI, and GPR are good predictors of patients who are more likely to progress to severe liver disease, and may be useful for risk stratification based on specific individual median splits.

2 | METHODS

From May 1991 to November 2016, 86 participants with CFLD who received a liver biopsy as part of routine clinical care or had explant histopathology were identified from two institutions: Texas Children's Hospital, Houston, Texas and Queensland Children's Hospital, Brisbane, Australia, 42 of which were included in the study. CFLD was defined a priori as subjects having ≥2 of the following: (1) hepatomegaly (defined as palpable on clinical exam or large for age on ultrasound) with or without splenomegaly; (2) a persistent (>6 months) elevation of serum alanine aminotransferase (ALT; level >1.5× upper limit of normal [ULN]); or (3) abnormal liver ultrasound (US) findings (abnormal echogenicity or a nodular edge), as we previously describe.[20,24,29] Participants with other causes of concomitant hepatobiliary disease were excluded. Other exclusion criteria included: lack of biopsy, missing laboratory values and missing clinical information.

Participants with a liver biopsy had a minimum of either a dual-pass 2 cm core of liver tissue or wedge biopsy for histologic evaluation. Biopsies were staged for hepatic fibrosis using Metavir classification[30] with F0-2 fibrosis classified as not severe and F3-4 classified as severe fibrosis. Liver and CF-specific clinical variables, liver biochemistries and anthropometrics were collected up to 5.8 years prior to liver biopsy in all subjects and used as "baseline" values for analyses. These values were again obtained within a 30-day goal window of subsequent liver biopsy and included platelet count, AST, ALT and GGT. FIB-4,[31] APRI,[32] and GPR,[33] calculated using an upper limit of normal of 40 U/L for AST and 19 U/L for GGT. Lastly, the change per year in platelets, AST, ALT, FIB-4, APRI, GGT and GPR were also assessed based on the time elapsed between the initial (baseline) visit and the time of subsequent labs (at biopsy). Spirometry values were obtained ±6 months of their associated baseline laboratory values. Abnormal baseline US findings were defined as splenomegaly, hepatomegaly, liver coarseness or nodularity, increased liver echogenicity, fatty liver infiltration, or abnormal doppler flow.

Patient demographics and clinical characteristics are summarized by medians with minimum and maximum values or frequencies with percentages. The Wilcoxon rank sum test or Fisher's exact test compared F0-2 versus F3-4. A multiple logistic regression model predicting F3-4 is estimated with change per year in FIB-4, APRI, PLT, and GPR as predictors. The model estimates the area under the ROC curve (95% CI). Kaplan–Meier curves estimate time-to-severe fibrosis, assuming F3-4 occurred at the time of biopsy. Participants who had not developed F3-4 were censored for this event at the time of biopsy. Median splits were used for continuous predictors, and the log-rank test compared cohorts. Statistical significance was assessed at the two-sided .05 level for all hypothesis tests without adjusting for multiple comparisons. Multiple logistic regression was

performed using predictors with 90% data completeness that were significant by Fisher's exact test. Median and 95% confidence intervals were derived from KM curves analysis. All analyses were completed using SAS for Windows version 9.4 (Cary, NC).

3 | RESULTS

A total of 42 participants (57% male) with CFLD who had received ≥1 liver biopsy (native or explant) met criteria for inclusion in the study (Figure S1). The median age at initial "baseline" lab collection and subsequent time of liver biopsy was 7.6 and 10.3 years, respectively. Baseline liver and CF-specific clinical variables, liver biochemistries and anthropometrics were collected over a median of 2.1 years (min .04, max 5.81) prior to liver biopsy. Each fibrosis stage using the METAVIR classification[27] (F0-F4) was represented in this study as follows for F0, F1, F2, F3, and F4: 14% ($n=6$), 19% ($n=8$), 19% ($n=8$), 19% ($n=8$) and 29% ($n=12$), respectively (Figure S2). Participants were categorized as non-severe, F0-2 ($n=22$) and severe, F3-4 ($n=20$). There were no significant differences in the distribution of sex, pancreatic sufficiency, CF genotype, history of meconium ileus, and isolation of *Pseudomonas aeruginosa* infection prior to the age of 2 years between the different stages of fibrosis (Table 1). No participants with F0 had CF-related diabetes mellitus (CFRD).

3.1 | Baseline differences between F0-2 and F3-4

Median age at baseline for participants later found to develop F3-4 was 10.5 years versus 5.8 years for F0-2 ($p=.08$, Table 1). Baseline BMI *z*-scores were not significantly different in F3-4 versus F0-2. Median FEV_1% predicted was lower at baseline in F3-4 compared with F0-2 (58.8% vs. 85%, $p=.03$). Exposure to ursodeoxycholic acid (UDCA) was higher in F3-4 versus F0-2 (100% vs. 68.2%, $p=.01$) and all F3-4 had a history of an abnormal liver ultrasound compared to F0-2 (100% vs. 72.7%, $p=.02$) (Table 2). There were no significant differences between groups in the frequency of documented hepatomegaly or splenomegaly, but both were more common among patients with F3-4. A majority of F3-4 had a clinical diagnosis of portal hypertension with a much lower prevalence in F0-2 (55% vs. 4.5%, $p<.001$). Liver biochemistries and biomarker indices were significantly elevated at baseline in participants with F3-4 versus F0-2, including GGT (59.5 U/L vs. 18 U/L, $p=.002$), APRI (.5 vs. .28, $p=.003$), FIB-4 (.23 vs. .11, $p=.03$), and GPR (.21 vs. .06, $p<.001$). ALT, AST, and platelet counts were not statistically different between cohorts. Only participants with F3-4 had documented oesophageal varices (33% vs. 0%; $p=.01$) (Table 2).

3.2 | Differences at time of final biopsy between F0-2 and F3-4

By the time of liver biopsy, age was not significantly different in F3-4 versus F0-2 (11.5 and 10.2 years, $p=.15$). Platelet count and liver biochemistries among F3-4 were significantly different versus F0-2 by the time of biopsy (Table 2). Median platelet count decreased by 127 $\times 10^3/\mu L$ in participants with F3-4 versus only 39 $\times 10^3/\mu L$ with F0-2 ($p=.008$). F3-4 also had significantly higher biomarker values at time of biopsy compared with F0-2: FIB-4 (.48 vs. .16, $p=.006$), APRI (.95 vs. .27, $p<.001$), and GPR (.33 vs. .06, $p<.001$) (Table 2).

TABLE 1 Baseline demographic, anthropometrics, and CF-specific variables.

	F0-2 ($n=22$)		F3-4 ($n=20$)		
Characteristic[b]	*N*	Summary statistic[a]	*N*	Summary statistic[a]	*p*-value[b]
Age at baseline visit (years)	22	5.8 (.1, 12.0)	20	10.5 (.0, 18.2)	.075
Male	13/22	59%	11/20	55%	>.999
Height (cm)[c]	18	127 (50, 155)	16	148 (58, 173)	**.022**
Weight (kg)[c]	18	23.9 (3.0, 41.0)	17	37.2 (2.8, 69.3)	**.044**
BMI *z*-score[c]	14	.0 (−1.6, 1.4)	14	.1 (−2.1, 1.3)	.982
FEV1% predicted[d]	13	85.2 (50, 110)	11	58.8 (27, 99)	**.002**
Pancreatic insufficiency	20/21	95%	17/19	89%	.596
CFRD	5/22	23%	8/20	40%	.320
History of meconium ileus	9/16	56%	7/13	54%	>.999
F508del homozygous	16/21	76%	11/17	65%	.394
Pseudomonas +<2 years	2/21	10%	2/12	17%	.610

[a]Summary statistics are calculated as % within group or medians with minimum and maximum values.

[b]Fisher's exact test for categorical variables or Wilcoxon rank sum test for continuous variables.

[c]Height, weight, and BMI *z*-score were measured at the baseline lab date (AST, ALT, PLT, GGT) except for 9 patients whose measures were observed within about 12-months of the baseline lab date (median −2.1, min −11.5, max 13.1).

[d]FEV1% predicted as measured at baseline lab date (AST, ALT, PLT, GGT) except for 13 patients whose measures were observed within about 12-months of baseline lab date (median .1, min −11.5, max 13.1).

Bold indicates significance level at *p*-value $p<.05$

TABLE 2 Liver-specific clinical variables.

Characteristic	F0-2 ($n=22$)		F3-4 ($n=20$)		
	N	%[a]	*N*	%[a]	*p*-value[b]
UDCA exposure (%)	15/22	68.2%	20/20	100%	**.009**
Abnormal US (%)	16/22	72.7%	20/20	100%	**.022**
Hepatomegaly (%)	7/22	31.8%	13/20	65%	.062
Splenomegaly (%)	6/22	27.3%	11/20	55%	.115
Portal hypertension (%)	1/22	4.5%	11/20	55%	**.0004**
Oesophageal varices (%)	0/19	0%	5/15	33.3%	**.011**
Lab value					
Platelets ($\times 10^3/\mu L$)					
Baseline	22	336.5 (232, 796)	20	302.5 (31–545)	.081
At biopsy[c]	22	297.5 (215, 634)	20	175.5 (20, 523)	**.008**
Change	22	−39 (−179, 211)	20	−25 (−265 230)	.783
Change per year	22	−18.1 (−10 889, 121)	20	−20.7 (−154.8, 770.2)	.626
ALT (units/L)					
Baseline	22	34 (21, 129)	20	42 (19, 100)	.960
At biopsy[c]	22	43.5 (20, 120)	20	46 (19, 171)	.96
Change	22	4.5 (−39, 95)	20	0 (−57, 132)	.454
Change per year	22	1.4 (−2068.3, 77.7)	20	.1 (−107.4, 170.8)	.591
AST (units/L)					
Baseline	22	38 (17, 95)	20	51.5 (23, 101)	.099
At biopsy[c]	22	42.5 (14, 66)	20	44.5 (14, 176)	.068
Change	22	−6.5 (−38, 28)	20	6.5 (−47, 95)	.248
Change per year	22	−2.2 (−1885.8, 15.7)	20	2.9 (−272, 304.7)	.326
GGT (units/L)					
Baseline	22	18 (7, 889)	18	59.5 (7, 264)	**.002**
At biopsy[c]	21	19 (10, 812)	20	39.5 (18, 469)	**.006**
Change	21	2 (−77, 60)	18	1 (−73, 205)	.635
Change per year	21	.6 (−4684.2, 19.7)	18	−2.3 (−343.5, 686.5)	.548
FIB-4					
Baseline	22	.1 (0, .2)	20	.2 (0, 6.7)	**.033**
At biopsy[c]	22	.2 (0, .3)	20	.5 (.0, 17.2)	**.006**
Change	22	0 (−.1, .1)	20	.1 (−.4, 13.7)	.447
Change per year	22	.0 (.0, .2)	20	.0 (−1.2, 9.5)	.338
APRI					
Baseline	22	.3 (.1, .6)	20	.5 (.2, 6.7)	**.003**
At biopsy[c]	22	.3 (.1, .7)	20	.9 (.1, 22)	**.0006**
Change	22	0 (−.3, .3)	20	.1 (−2.4, 17.7)	.248
Change per year	22	.0 (−.2, 2.2)	20	.0 (−3.9, 12.2)	.462
GPR					
Baseline	22	.1 (.0, 2.1)	18	.2 (.0, 4.6)	**.0008**
At biopsy[c]	21	.1 (.0, 3.2)	20	.3 (.1, 9.7)	**.0004**
Change	21	0 (−.1, 1.2)	18	0 (−.9, 5.1)	.625
Change per year	21	.0 (.0, 71)	18	.0 (−2.2, 3.5)	.567

Abbreviations: ALT, aspartate aminotransferase; APRI, [(AST/upper limit of normal AST) ×100]/platelet count (10^9/L); AST, alanine aminotransferase; FIB-4, (age (years)×AST [U/L])/(platelets (10^9/L)×(√ALT [U/L]); GGT, gamma-glutamyl transferase; GGT PR, gamma-glutamyl transferase to platelet ratio; UDCA, ursodeoxycholic acid.

[a]Summary statistics are calculated as % within group or medians with minimum and maximum values.

[b]Fisher's exact test for categorical variables or Wilcoxon rank sum test for continuous variables.

[c]Lab measures were observed within 24 months prior to the biopsy date (median 0, min = −23.9, max = 0).

3.3 | Time to development of severe fibrosis (F3-4) in cystic fibrosis-related liver disease

Participant data was analysed via Kaplan–Meier, product-limit survival estimates to determine time to progression to F3-4 based on clinical variables. Participants who progressed to F3-4 did so in a median time of 3.3 years (95% CI [2.01, 4.5]) from baseline.

3.3.1 | Influence of baseline CF-specific variables

Certain CF-specific variables were found to be associated with time to develop F3-4 (Figure 1). Participants with a baseline FEV_1% predicted <64% progressed to F3-4 in a median of 1.05 years (95% CI: .36, 1.52) compared to 3.62 years (95% CI: 2.01, ∞) in those with FEV_1 >64%. Those participants who isolated *P. aeruginosa* from a respiratory culture prior to the age of 2 years, progressed to F3-4 in a median of 2.73 years (95% CI [.53, ∞]; $p = .04$,) compared to 3.78 years (95% CI [3.17, ∞]) in those who did not. Participants with a F508del heterozygous genotype progressed to F3-4 more rapidly ($p = .02$) than those with a F508del homozygous genotype with a median of 2.38 years (95% CI [.77, ∞]) and 3.62 years (95% CI [2.73, ∞]), respectively. Those with pancreatic insufficiency progressed slower to F3-4 ($p = .051$) with a median time of 3.62 years (95% CI [2.01, ∞]) than those who were pancreatic sufficient (1.17 years (95% CI [.77, ∞]). There was no statistically significant difference in time to severe fibrosis for the variables of sex, history of meconium ileus, baseline age or BMI.

3.3.2 | Influence of baseline liver-specific variables

Liver-specific clinical variables were also associated with time to progression to F3-4. Participants with hepatomegaly ($p = .006$) and splenomegaly ($p < .001$) developed F3-4 sooner than those without. With hepatomegaly, the median time to F3-4 was 2.38 years (95% CI [.79, 3.62]) versus 4.45 years (95% CI [3.17, ∞]) in those without (Figure 1). Participants with splenomegaly had a median time to F3-4 of 1.17 years (95% CI [.77, 1.78]), compared to 3.78 years (95% CI [3.17, ∞]) in those with normal spleen size. Participants who never had an abnormal liver ultrasound never progressed to F3-4. Those with a history of an abnormal ultrasound however progressed to F3-4 in a median of 3.17 years (95% CI [1.52, 3.78]; $p = .02$). The presence of oesophageal varices was also associated with a more expedited progression to F3-4 ($p = .001$) in a median time of 1.52 years (95% CI [.77, ∞]). Those without varices developed F3-4 in a median time of 4.45 years (95% CI [3.17, ∞]). The presence of portal hypertension was likewise associated with faster progression to F3-4 ($p < .0001$). Those with portal hypertension ($n = 12$) developed F3-4 in 1.05 (95% CI [.36, 1.52]) years versus 4.45 years (95% CI [3.17, ∞]) in those without. One F2, one F3, and ten F4 participants had portal hypertension. There were no significant differences in time to severe fibrosis in participants who had been exposed to UDCA ($p = .054$), though severe fibrosis did not occur in any participant who was not exposed to UDCA.

3.3.3 | Influence of baseline liver biochemistries and biomarker indices on time to progression

Several baseline labs were associated with earlier progression to F3-4 (Figure 1). Participants with a platelet count <329.5 $\times 10^3/\mu L$ progressed to F3-4 in a median of 1.78 years (95% CI [1.05, ∞]) compared to 3.62 years (95% CI: 2.73, ∞) in those with platelets ≥329.5 $\times 10^3/\mu L$ ($p = .20$). AST of ≥39 units/L was associated with more rapid development of F3-4 (2.73 vs. 4.45 years, $p = .08$). There was no significant difference in time to severe fibrosis based on ALT. There was a significant difference in time to F3-4 between those with high GGT (≥25.5 units/L) versus lower GGT (<25.5 units/L). The median time to F3-4 for those with high GGT was 1.78 years (.96, 3.17). The median time for those with lower GGT values was not estimable, but the lower bound of the 95% CI was 3.78 years. FIB-4 ≥.13 was associated with F3-4 development (1.78 vs. 4.45 years, $p = .008$). Participants with an APRI ≥.36 progressed to F3-4 more rapidly (1.78 vs. 4.45 years, $p = .005$). Those with a GPR ≥.09 progressed to F3-4 ($p < .001$) in a median of 1.52 years (95% CI [.96, 3.17]).

3.4 | Predicting severe fibrosis (F3-4) in cystic fibrosis-related liver disease over time

Logistic regression and receiver operating characteristic curve analysis estimated the association between the change per year in APRI, FIB-4, platelet count and GPR with fibrosis stage (F0-2 vs. F3-4) (Table 2). There were no significant associations in change per year of FIB-4, APRI, GPR or platelet count, respectively with the development of F3-4 (Table 2, Figure 2). However, when change in APRI, FIB-4 and GPR were simultaneously included in a multiple logistic regression model, the AUROC to predict F3-4 was .81 ($p < .0001$, 95% CI [.66, .96]) (Figure 3).

4 | DISCUSSION

The ability to identify CF patients at risk of developing severe liver disease and the timing of when to more closely monitor hepatic deterioration has been a challenge for paediatric subspecialty providers. Previous studies have reported varying risk factors for the development of significant liver disease[4,5,11,15,17,34] but few studies exist that have been validated by liver biopsy. In the present study, we identify differences in clinical parameters that are evident a median of two years prior to biopsy in those participants with CFLD who progress to severe fibrosis. Interestingly, participants in our study who developed F3-4 fibrosis had moderate lung disease at baseline, as defined by the American Thoracic Society (ATS) as an FEV_1 predicted of 64% or less,[35] while those with F0-2 on biopsy

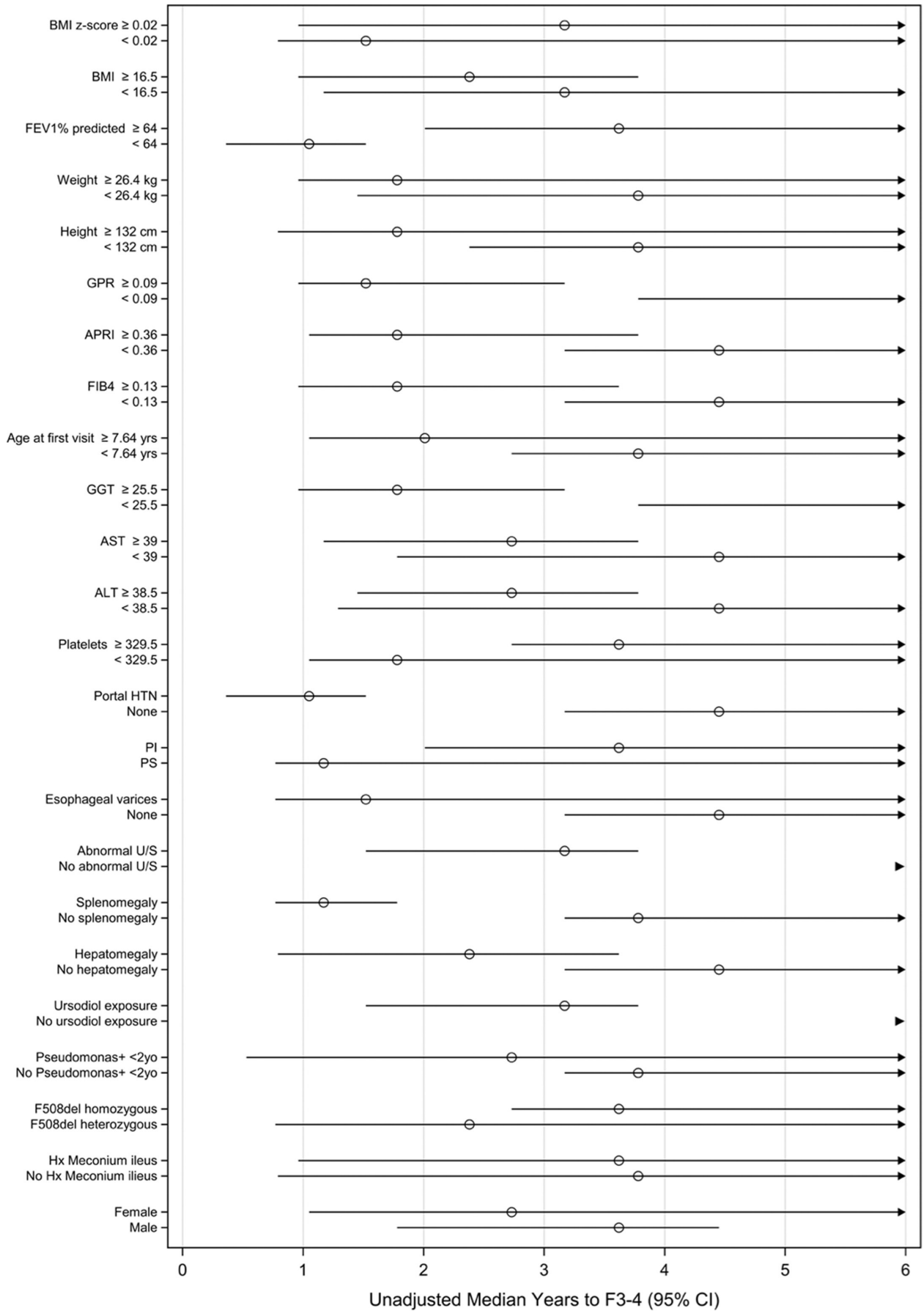

FIGURE 1 Unadjusted median years (95% CI) to severe fibrosis estimated by Kaplan–Meier curves. Median split used for continuous variables.

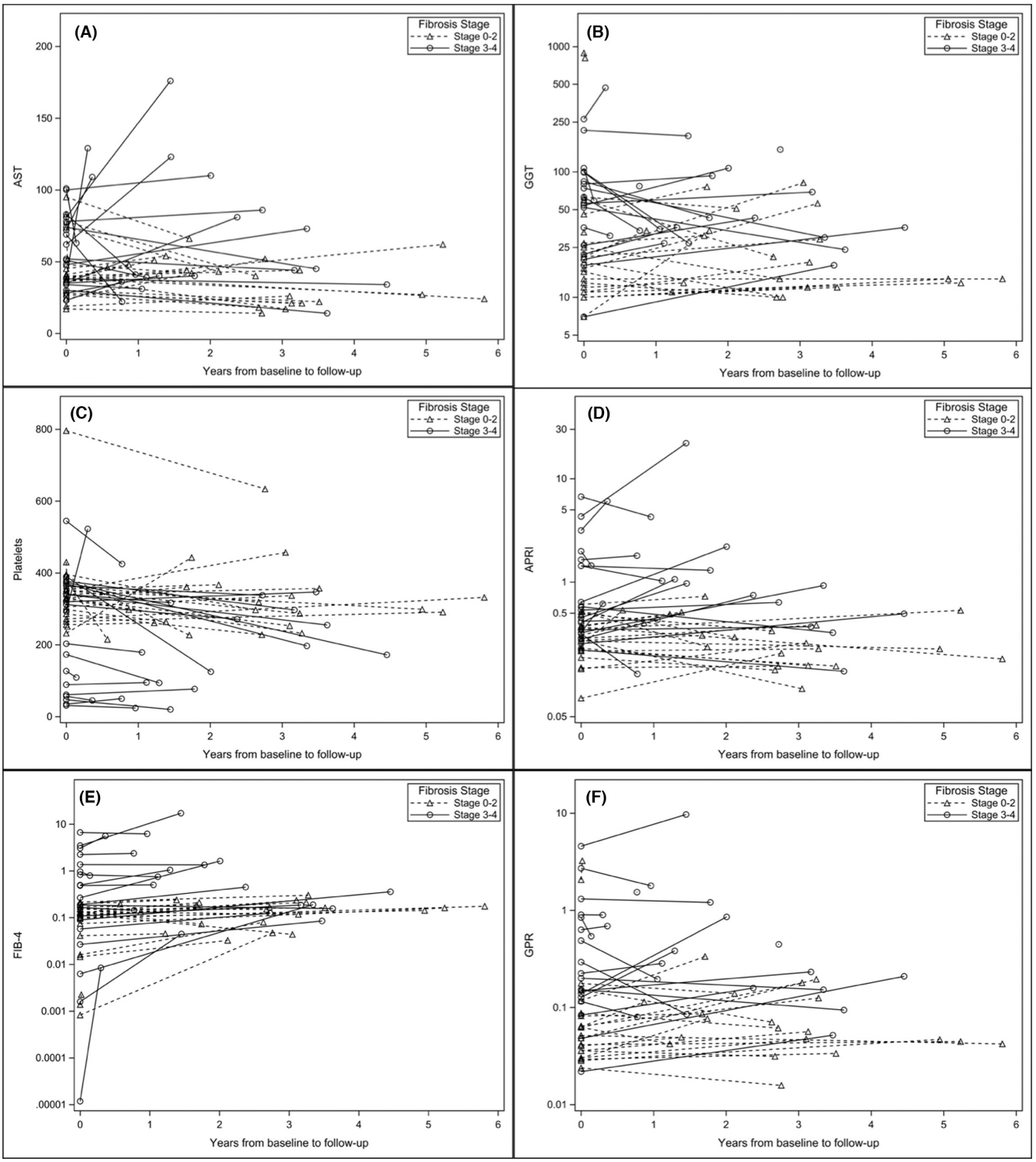

FIGURE 2 Change per year in liver-specific clinical variables.

had normal lung function. Prior studies have reported varied severity of lung disease in participants with CFLD, with few including liver histology. Colombo et al. reported that lung function is no worse in patients with CFLD[4] and data from Polileni et al. suggest that pulmonary decline is not accelerated among those with CFLD versus those without.[36] Our data suggest the rate of progression to severe fibrosis in CFLD patients with moderate lung function impairment[35] using a split of FEV_1 <64% was 3-fold faster than those patients with normal lung function.[4,36,37]

Surveillance for development of CFLD often involves monitoring liver biochemistries.[3] In the last several years, calculated biomarker indices such as FIB-4,[6,13,24] APRI[6,13,16,20,24] and GPR[28] have demonstrated significant potential in the detection or fibrosis staging of CFLD. Our study reveals that several of these labs and biomarker indices including

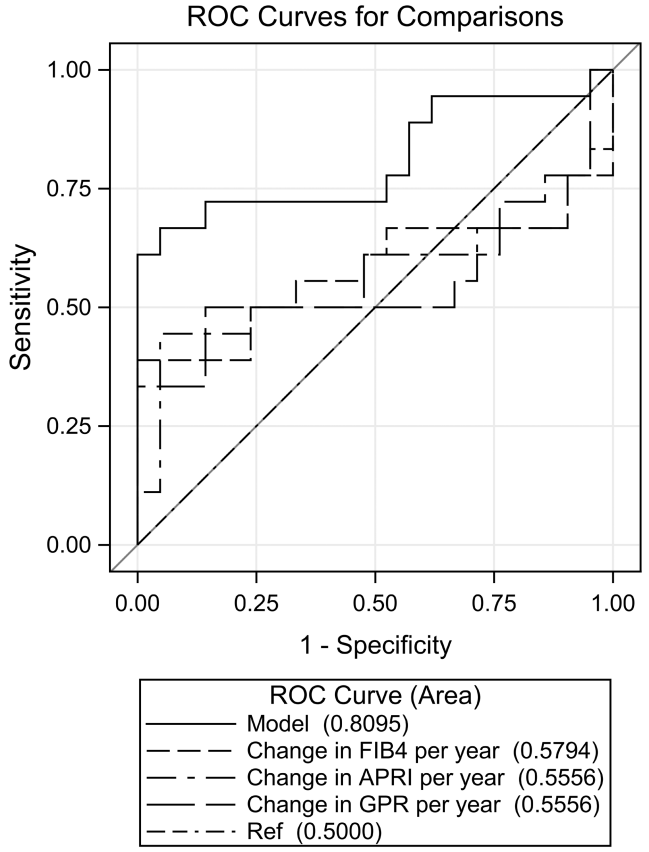

FIGURE 3 Area under the receiver operating characteristic curves (AUROC) for change in FIB4 per year, change in APRI per year, and change in GPR per year.

GGT, FIB-4, APRI and GPR are abnormally elevated in the years leading up to biopsy, and continue to worsen at the time of liver biopsy. In the present study, participants with F3-4 had a higher baseline APRI in the 2 years prior to biopsy. This indicates a higher threshold may be useful to further categorize early risk for development of severe fibrosis and is consistent with our prior work which identified an APRI threshold of .462 to be associated with F3-4.[24] Interestingly, even a modest elevation in AST is associated with a >2-fold increased rate of progression to severe fibrosis. Likewise, GGT also showed an association with 3-fold more rapid progression to F3-4 in those whose values >68.3 units/L. Notably, median platelet count at the time of biopsy among F3-4 had dropped more than 40% from baseline just 2 years prior, though was still within normal limits. Further, we found that even platelet counts that are normal by laboratory standards can be useful to assess risk for severe fibrosis, and a rapid or profound decrease in platelet counts should raise concern for emerging CFLD and portal hypertension.

We anticipated that the change in clinical biomarkers (FIB-4, APRI and GPR) over time would be useful in predicting F3-4 development. While a limited sample size over a relatively short time period precluded demonstrating statistical significance when assessed individually, the combination of these biomarkers when assessed as change per year, proved particularly useful in predicting F3-4. These values are easily obtained and calculated, and could be included in yearly CF laboratory evaluations.

Limitations of our study include both a small sample size and number of laboratory measures over time. We excluded 44 patients from our analysis due to lack of documented liver biopsy or missing lab values. Additionally, liver biopsy is not a standard of care in patients with CFLD and therefore could not be protocolized. Hence, we acknowledge the likelihood of referral bias as this study includes only participants in which the provider determined that an initial liver biopsy was warranted. Our data set also includes a large number of liver samples from the US cohort being from explants (6 out of 16 patients), introducing the potential for self-selection bias for patients with a more aggressive or severe clinical phenotype. However, this study represents one of the largest paediatric cohorts assessing both baseline and change in biomarker indices over time based on real life clinical care in children with varying stages of CFLD, as validated by liver biopsy. As our data spanned a 17-year time period, data missingness due to transition from paper charting to electronic health record systems precluded our ability to power the study as desired. Additionally, given the retrospective design, not all missing data elements could be reconciled. The frequency of laboratory and pulmonary function tests was dictated by real-time clinical care and likely reflects varying severity of disease or varying clinical practices. In evaluating time to progression to F3-4 fibrosis, we were limited by our assumption that these participants did not already have severe liver fibrosis at the time of their baseline labs or that participants did not develop severe fibrosis until time of their liver biopsy. This was unable to be confirmed due to lack of biopsy data at baseline.

Infection with *P. aeruginosa* from a respiratory culture prior to the age of 2 years was found to be associated with a more rapid progression to F3-4 as well. This raises the question of whether earlier antibiotic exposure, earlier infection or longer states of inflammation modify the risk of developing fibrosis or portal hypertension via mechanisms such as nodular regenerative hyperplasia.[38] In a prior large multi-center study, it was reported that earlier *P. aeruginosa* infection may be protective from development of abnormal liver ultrasound patterns among children with CF,[39] making our observations of particular interest.

The more rapid progression to F3-4 in participants who were F508del heterozygous or pancreatic sufficient (PS) was also unexpected. In our study, children with PS demonstrated a 3-fold more rapid rate of progression to F3-F4 liver disease while others have shown that those who develop nodular appearing livers have an increased incidence of pancreatic insufficiency.[23,40] This discrepant finding could either be explained by our small sample size, or alternatively, consideration that what was categorized as "cirrhosis" by imaging in other studies was not histologic cirrhosis, as demonstrated on liver biopsy validation in our study. It would be reasonable to postulate that a heterozygote with some residual CFTR function may not develop as severe liver disease,[40] though it is important to note that degree of fibrosis is not equivalent to degree of nodularity or portal hypertension.

Importantly, not a single participant in our cohort who had a normal abdominal US developed F3-4, further supporting the utility of ultrasound as an important screening measure and clinical predictor.

In summary, this study provides a unique assessment of the clinical and biochemical variables over time that are associated with the development of liver biopsy-validated severe paediatric CFLD, including moderate pulmonary dysfunction defined as FEV_1% <64%. For patients identified early as more rapid progressors, there is emerging evidence from retrospective studies of the potential benefit of CFTR modulators to improve liver biomarkers or even reduce the incidence of cirrhosis in those with CFLD.[41,42] In the future, targeted gene therapy or other proposed medications that promote bile flow or reduce portal pressures could also be considered.[43] We also found a negative association between severe liver-biopsy proven fibrosis with pancreatic insufficiency and F508del homozygosity which merits further study and validation by others. Importantly, we have identified specific threshold values (FIB-4 ≥.55, APRI ≥.78, GPR ≥.41) that differentiate patients at higher and more rapid risk for development of F3-4 up to a median of 2 years prior to liver biopsy. These differences may better assist in risk-stratifying and monitoring patients for CFLD, allowing for earlier surveillance of disease progression and intervention when available.

CONFLICT OF INTEREST STATEMENT

The authors do not have any disclosures to report.

ETHICS STATEMENT

This study was approved by the Baylor College of Medicine Institutional Review Board. This material is the authors' own original work, which has not been previously published elsewhere. The paper is not currently being considered for publication elsewhere.

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REFERENCES

1. Flass T, Narkewicz MR. Cirrhosis and other liver disease in cystic fibrosis. *J Cyst Fibros*. 2013;12(2):116-124.
2. Debray D, Kelly D, Houwen R, Strandvik B, Colombo C. Best practice guidance for the diagnosis and management of cystic fibrosis-associated liver disease. *J Cyst Fibros*. 2011;10(suppl. 2):S29-S36.
3. Sokol RJ, Durie PR. Recommendations for management of liver and biliary tract disease in cystic fibrosis. Cystic Fibrosis Foundation hepatobiliary disease consensus group. *J Pediatr Gastroenterol Nutr*. 1999;28(suppl. 1):S1-S13.
4. Colombo C, Battezzati PM, Crosignani A, et al. Liver disease in cystic fibrosis: a prospective study on incidence, risk factors, and outcome. *Hepatology*. 2002;36(6):1374-1382.
5. Lindblad A, Glaumann H, Strandvik B. Natural history of liver disease in cystic fibrosis. *Hepatology*. 1999;30(5):1151-1158.
6. Stonebraker JR, Ooi CY, Pace RG, et al. Features of severe liver disease with portal hypertension in patients with cystic fibrosis. *Clin Gastroenterol Hepatol*. 2016;14(8):1207-1215.e3.
7. 2017 CF Foundation Participant Registry.
8. Lewindon PJ, Shepherd RW, Walsh MJ, et al. Importance of hepatic fibrosis in cystic fibrosis and the predictive value of liver biopsy. *Hepatology*. 2011;53(1):193-201.
9. Debray D, Corvol H, Housset C. Modifier genes in cystic fibrosis-related liver disease. *Curr Opin Gastroenterol*. 2019;35(2):88-92.
10. Bartlett JR, Friedman KJ, Ling SC, et al. Genetic modifiers of liver disease in cystic fibrosis. *Jama*. 2009;302(10):1076-1083.
11. Colombo C, Apostolo MG, Ferrari M, et al. Analysis of risk factors for the development of liver disease associated with cystic fibrosis. *J Pediatr*. 1994;124(3):393-399.
12. Baldissera M, Lewindon PJ, Ramm LE, Hartel GF, Mattiello R, Ramm GA. Advanced but not mild liver disease is a predictor of decreased survival in children with cystic fibrosis, with far greater impact in females: a 27-year real-life cohort study. *J Cyst Fibros*. 2022;21(2):230-235.
13. Leung DH, Narkewicz MR. Cystic fibrosis-related cirrhosis. *J Cyst Fibros*. 2017;16(suppl. 2):S50-S61.
14. Moyer K, Balistreri W. Hepatobiliary disease in patients with cystic fibrosis. *Curr Opin Gastroenterol*. 2009;25(3):272-278.
15. Gominon AL, Frison E, Hiriart JB, et al. Assessment of liver disease progression in cystic fibrosis using transient elastography. *J Pediatr Gastroenterol and Nutr*. 2018;66(3):455-460.
16. Lewindon PJ, Puertolas-Lopez MV, Ramm LE, et al. Accuracy of transient elastography data combined with APRI in detection and staging of liver disease in pediatric participants with cystic fibrosis. *Clin Gastroenterol Hepatol*. 2019;17(12):2561-2569.e5.
17. Klotter V, Gunchick C, Siemers E, et al. Assessment of pathologic increase in liver stiffness enables earlier diagnosis of CFLD: results from a prospective longitudinal cohort study. *PLoS ONE*. 2017;12(6):e0178784.
18. Aqul A, Jonas MM, Harney S, et al. Correlation of transient elastography with severity of cystic fibrosis-related liver disease. *J Pediatr Gastroenterol Nutr*. 2017;64(4):505-511.
19. Calvopina DA, Noble C, Weis A, et al. Supersonic shear-wave elastography and APRI for the detection and staging of liver disease in pediatric cystic fibrosis. *J Cyst Fibros*. 2020;19(3):449-454.
20. Calvopina DA, Chatfield MD, Weis A, et al. MicroRNA sequencing identifies a serum microRNA panel, which combined with aspartate aminotransferase to platelet ratio index can detect and monitor liver disease in pediatric cystic fibrosis. *Hepatology*. 2018;68(6):2301-2316.
21. Cook NL, Pereira TN, Lewindon PJ, Shepherd RW, Ramm GA. Circulating microRNAs as noninvasive diagnostic biomarkers of liver disease in children with cystic fibrosis. *J Pediatr Gastroenterol Nutr*. 2015;60(2):247-254.
22. Schmid P, Bregenzer A, Huber M, et al. Progression of liver fibrosis in HIV/HCV Co-infection: a comparison between non-invasive assessment methods and liver biopsy. *PLoS ONE*. 2015;10(9):e0138838.
23. Friedrich-Rust M, Schlueter N, Smaczny C, et al. Non-invasive measurement of liver and pancreas fibrosis in participants with cystic fibrosis. *J Cyst Fibros*. 2013;12(5):431-439.
24. Leung DH, Khan M, Minard CG, et al. Aspartate aminotransferase to platelet ratio and fibrosis-4 as biomarkers in biopsy-validated pediatric cystic fibrosis liver disease. *Hepatology*. 2015;62(5):1576-1583.
25. Li Q, Lu C, Li W, Huang Y, Chen L. The gamma-glutamyl transpeptidase to platelet ratio for non-invasive assessment of liver fibrosis in participants with chronic hepatitis B and non-alcoholic fatty liver disease. *Oncotarget*. 2017;8(17):28641-28649.
26. Liu DP, Lu W, Zhang ZQ, et al. Comparative evaluation of GPR versus APRI and FIB-4 in predicting different levels of liver fibrosis of chronic hepatitis B. *J Viral Hepat*. 2018;25(5):581-589.
27. Sellers ZM, Lee LW, Barth RA, Milla C. New algorithm for the integration of ultrasound into cystic fibrosis liver disease screening. *J Pediatr Gastroenterol Nutr*. 2019;69(4):404-410.
28. Calvopina DA, Lewindon PJ, Ramm LE, et al. Gamma-glutamyl transpeptidase-to-platelet ratio as a biomarker of liver disease and hepatic fibrosis severity in paediatric cystic fibrosis. *J Cyst Fibr*. 2022;21(2):236-242.
29. Bedossa P, Poynard T, The French METAVIR Cooperative Study Group. An algorithm for grading activity in chronic hepatitis C. *Hepatology*. 1996;24:289-293.

30. Sterling RK, Lissen E, Clumeck N, et al. Development of a simple noninvasive index to predict significant fibrosis in participants with HIV/HCV coinfection. *Hepatology*. 2006;43(6):1317-1325.
31. Wai CT, Greenson JK, Fontana RJ, et al. A simple noninvasive index can predict both significant fibrosis and cirrhosis in participants with chronic hepatitis C. *Hepatology*. 2003;38(2):518-526.
32. Lemoine M, Shimakawa Y, Nayagam S, et al. The gamma-glutamyl transpeptidase to platelet ratio (GPR) predicts significant liver fibrosis and cirrhosis in participants with chronic HBV infection in West Africa. *Gut*. 2016;65(8):1369-1376.
33. Bodewes FAJA, van der Doef HPJ, Houwen RHJ, Verkade HJ. Increase of serum γ-glutamyltransferase associated with development of cirrhotic cystic fibrosis liver disease. *J Pediatr Gastroenterol Nutr*. 2015;61(1):113-118.
34. Pellegrino R, Viegi G, Brusasco V, et al. Interpretative strategies for lung function tests. *Eur Respir J*. 2005;26(5):948-968.
35. Polineni D, Piccorelli AV, Hannah WB, et al. Analysis of a large cohort of cystic fibrosis participants with severe liver disease indicates lung function decline does not significantly differ from that of the general cystic fibrosis population. *PLoS ONE*. 2018;13(10):e0205257.
36. Miller MR, Sokol RJ, Narkewicz MR, Sontag MK. Pulmonary function in individuals who underwent liver transplantation: from the US cystic fibrosis foundation registry. *Liver Transpl*. 2012;18(5):585-593.
37. Wu H, Vu M, Dhingra S, et al. Obliterative portal venopathy without cirrhosis is prevalent in pediatric cystic fibrosis liver disease with portal hypertension. *Clin Gastroenterol Hepatol*. 2019;17:2134-2136.
38. Leung DH, Ye W, Molleston JP, et al. Baseline ultrasound and clinical correlates in children with cystic fibrosis. *J Pediatr*. 2015;167(4):862-868.e2.
39. Nascimento FD, Sena NA, Ferreira TD, Marques CD, Silva LR, Souza EL. Hepatobiliary disease in children and adolescents with cystic fibrosis. *J Pediatr (Rio J)*. 2018;94(5):504-510.
40. Veit G, Avramescu RG, Chiang AN, et al. From CFTR biology toward combinatorial pharmacotherapy: expanded classification of cystic fibrosis mutations. *Mol Biol Cell*. 2016;27(3):424-433.
41. Levitte S, Fuchs Y, Wise R, Sellers ZM. Effects of CFTR modulators on serum biomarkers of liver fibrosis in children with cystic fibrosis. *Hepatol Commun*. 2023;7(2):e0010.
42. Ramsey ML, Wellner MR, Porter K, et al. Cystic fibrosis patients on cystic fibrosis transmembrane conductance regulator modulators have a reduced incidence of cirrhosis. *World J Hepatol*. 2022;14(2):411-419.
43. Staufer K. Current treatment options for cystic fibrosis-related liver disease. *Int J Mol Sci*. 2020;21(22):8586.

SUPPORTING INFORMATION

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

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