

# Biopsy Validated Study of Biomarkers for Liver Fibrosis and Transplant Prediction in Inherited Cholestasis

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Alagille syndrome (ALGS) and progressive familial intrahepatic cholestasis (PFIC) are inherited cholestatic disorders with risk of developing end-stage liver disease requiring liver transplantation (LT). We investigated aspartate aminotransferase-to-platelet ratio index (APRI), Fibrosis-4 score (FIB-4), and conjugated bilirubin as biomarkers to assess fibrosis severity and risk for LT among children with ALGS and PFIC. This multicenter, cross-sectional study included 64 children with ALGS or PFIC (per genetics or strict clinical criteria) with APRI, FIB-4, and conjugated bilirubin levels collected within  $\pm 90$  days of their most recent liver biopsy. A single, blinded pathologist staged all biopsies (metavir; F0-F2: nonsevere, F3-F4: severe). Logistic regression and area under the receiver operating characteristic curve analysis (AUC) were used to assess biomarker associations with fibrosis severity and risk for LT. In ALGS, only APRI distinguished F3-F4 (AUC 0.72,  $P = 0.012$ ), with a cutoff greater than 2.97 demonstrating a sensitivity of 61.5% (95% confidence interval 0.32, 0.86) and specificity of 81.5% (0.62, 0.94). In ALGS, a 50% increase of APRI increased the odds of F3-F4 by 1.31-fold (1.04, 1.65;  $P = 0.023$ ). In ALGS, APRI (AUC 0.87;  $P < 0.001$ ) and FIB-4 (AUC 0.84;  $P < 0.001$ ) were able to predict risk for LT. In PFIC, only APRI distinguished F3-F4 (AUC 0.74,  $P = 0.039$ ), with a cutoff greater than 0.99 demonstrating a sensitivity of 80% (0.44, 0.98) and specificity of 64.3% (0.35, 0.87). In PFIC, only FIB-4 was able predict risk for LT (AUC 0.80;  $P = 0.002$ ). In ALGS or PFIC, conjugated bilirubin could not distinguish F3-F4 or predict risk for LT. **Conclusion:** This liver biopsy-validated study suggests that APRI is able to distinguish F3-F4 from F0-F2 in ALGS and PFIC. APRI and FIB-4 may also serve as predictors of risk for LT in ALGS (APRI and FIB-4) and PFIC (FIB-4). (*Hepatology Communications* 2020;4:1516-1526).

**A**lagille syndrome (ALGS) and progressive familial intrahepatic cholestasis (PFIC) represent distinct but rare inherited cholestatic disorders that display wide variability in degrees of liver fibrosis, progression of disease, and time to transplant.<sup>(1-8)</sup> Although genetic analysis is emphasized for either diagnosis, percutaneous liver biopsy has historically been an essential tool for histologic staging

and monitoring progression of disease.<sup>(9,10)</sup> Risks of liver biopsies are relatively low; however, there is still potential for bleeding, patient discomfort, infection, and side effects of anesthesia.<sup>(10,11)</sup>

An autosomal dominant disorder, ALGS is characterized by bile duct paucity and often presents with extrahepatic clinical features such as vertebral abnormalities, renal or cardiac disease, posterior embryotoxon,

*Abbreviations:* ALGS, Alagille syndrome; ALT, alanine aminotransferase; APRI, aspartate aminotransferase-to-platelet ratio index; AST, aspartate aminotransferase; AUC, area under the receiver operator characteristic curve; BA, biliary atresia; cBili, conjugated bilirubin; CFLD, cystic fibrosis liver disease; CI, confidence interval; FIB-4, fibrosis-4 score; GGT, gamma-glutamyl transferase; GPR, GGT-to-platelet ratio; IQR, interquartile range; LT, liver transplantation; PFIC, progressive familial intrahepatic cholestasis; ROC, receiver operator characteristic.

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or syndromic facies<sup>(4,12)</sup> with an estimated frequency of 1 in 30,000 to 50,000 live births per year.<sup>(5)</sup> Although liver disease may improve with age,<sup>(3)</sup> an estimated 43.5%-76% of patients with ALGS will undergo liver transplantation (LT) by adulthood.<sup>(1,13,14)</sup> PFIC consists of multiple disease subtypes, each characterized by various defects in transport of bile constituents<sup>(15,16)</sup> with autosomal recessive inheritance, and an estimated incidence of 1 in 50,000 to 100,000 live births per year.<sup>(15,17,18)</sup> The clinical course of liver disease in PFIC is sometimes rapid, representing up to 10%-15% of all childhood liver transplants.<sup>(15,19)</sup>

Developed initially to monitor the progression of liver fibrosis in adults with hepatitis C,<sup>(20,21)</sup> the simple biomarker indices aspartate aminotransferase (AST)-to-platelet ratio (APRI) and Fibrosis-4 score (FIB-4) use standard liver biochemistries. These indices have since been biopsy-validated for use in distinguishing liver fibrosis severity in pediatric hepatobiliary disorders such as biliary atresia (BA)<sup>(22)</sup> and cystic fibrosis liver disease (CFLD).<sup>(23)</sup> Total bilirubin and gamma-glutamyl transferase (GGT)-to-platelet ratio (GPR) have also been shown to be useful biomarkers of outcome in children with BA (transplant-free survival, death)<sup>(24)</sup> and CFLD (liver fibrosis detection).<sup>(25)</sup>

The primary objective of this study was to investigate the utility of APRI, FIB-4, and conjugated bilirubin as biomarkers of liver fibrosis validated by liver biopsy in children with ALGS and PFIC. Secondary objectives include investigation of the change in biomarker scores over time and assessment of biomarkers as predictors of risk for LT.

## Materials and Methods

This study was a multicenter, liver biopsy-validated, cross-sectional study examining the utility of APRI, FIB-4, and conjugated bilirubin as surrogates of fibrosis in children with genetically or clinically confirmed ALGS or PFIC. We also performed a longitudinal secondary analysis to track the chronological progression of biomarkers and analyzed biomarkers for prediction of risk for future LT. This study was approved by institutional review boards at each center or granted a waiver due to minimal risk. For inclusion, subjects were required to have at least one liver biopsy (from either native liver or explant) and documented genetic or clinical confirmation of ALGS or PFIC subtype 1-3 diagnosis. The diagnosis of ALGS or PFIC was first confirmed by documented genetic mutation (*JAG-1* or *NOTCH-2* for ALGS; *ATP8B1* for PFIC subtype 1; *ABCB11* for PFIC subtype 2; and *ABCB4* for PFIC subtype 3). If no records of genetic mutation were available, clinical criteria were used to confirm the diagnosis. Clinical criteria for ALGS included any combination of three or more of the following: (1) peripheral pulmonary stenosis (or tetralogy of Fallot), (2) posterior embryotoxon, (3) butterfly vertebrae, (4) renal manifestations, (5) vascular anomalies, (6) facial features historically described in ALGS,<sup>(4)</sup> or (7) or bile duct paucity (per pathologic reports). Clinical criteria for PFIC subtypes included the following: (1) PFIC subtype 1 = history of low/normal GGT and electron microscopy with Byler's bile; (2) PFIC

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subtype 2 = history of low/normal GGT and absent bile salt export pump protein staining or giant cell hepatitis; and (3) PFIC subtype 3 = high GGT with bile duct proliferation and a regular biliary tree (per imaging).

Liver biopsy cores were a minimum of 1 cm in length with 6-10 portal tracts to ensure sample adequacy (range of 1-7 cores per sample). Otherwise, tissue samples were from explants. Histological slides (trichrome/hematoxylin and eosin) were digitalized and reviewed by a single pediatric hepatopathologist who was blinded to all participant information. Each biopsy was staged for fibrosis using the metavir classification (F0 = no fibrosis, F1 = portal fibrosis without septa, F2 = portal fibrosis with few septa, F3 = numerous septa without cirrhosis, and F4 = cirrhosis). For this study, metavir stages 0-2 were classified as “nonsevere” fibrosis (F0-F2) and metavir stages 3-4 were classified as “severe” fibrosis (F3-F4). The most recent liver specimen was designated as the “final biopsy” and used for correlation with APRI, FIB-4, and conjugated bilirubin in the primary cross-sectional analysis. For participants with multiple biopsies ( $n = 16$ ), histology preceding the final biopsy were individually staged and correlated to biomarkers in a secondary longitudinal analysis. Participants who had an increase of at least one metavir stage of fibrosis over multiple biopsies were categorized as “progressors”; participants without progression in fibrosis over multiple biopsies were categorized as “nonprogressors.”

Labs (including conjugated bilirubin and GGT) were collected within  $\pm 90$  days of the biopsy. For explant biopsies, labs were collected at least 90 days preceding the biopsy. Age in years at the time of lab collection was used for FIB-4. For purposes of this study, the upper limit of normal AST was 30 U/L and the upper limit of normal GGT was 40 U/L.

## SIMPLE BIOMARKER INDICES CALCULATIONS

$$\text{APRI} = \frac{\text{AST[U/L]}/\text{upper limit of normal AST}}{\text{Platelet Count [10}^9\text{/L]}} \times 100$$

$$\text{FIB-4} = \frac{\text{Age (years)} \times \text{AST[U/L]}}{\text{Platelets [10}^9\text{/L]} \times \sqrt{\text{ALT[U/L]}}}$$

$$\text{GPR} = \frac{\text{GGT[U/L]}/\text{upper limit of normal GGT}}{\text{Platelet Count [10}^9\text{/L]}} \times 100$$

## STATISTICS

Patient and clinical characteristics are summarized using mean with SDs, median with 25th and 75th percentiles, and frequency with percentages. The summary statistics are stratified by fibrosis severity and compared using two-sample  $t$  test, Wilcoxon rank sum test, Fisher's exact test, or chi-square test. Logistic regression accounting for the within-hospital correlation was used to assess the association between fibrosis severity and log-transformed APRI, FIB-4, and conjugated bilirubin. For each predictor, a receiver operating characteristic (ROC) analysis with area under the curve (AUC) was performed. The ROC curves were compared. All analyses were performed using Stata v.15 software (College Station, TX).

## Results

### OVERALL FINDINGS

This study included 64 individual participants with either ALGS ( $n = 40$ ) or PFIC subtypes 1-3 ( $n = 24$ , Supporting Table S1) with liver biopsies performed between January 2003 to December 2015. In total, 33 (51.6%) participants were confirmed genetically ( $JAG1 = 16$ ,  $NOTCH2 = 1$ ,  $ATP8B1 = 7$ ,  $ABCB11 = 5$ ,  $ABCB4 = 4$ ), and 30 (46.9%) met strict clinical criteria for either ALGS or PFIC. One participant with PFIC subtype 2 was included based on clinical history of intracellular cholestasis with low GGT and  $ABCB11$  heterozygosity. The overall median age at liver biopsy for all participants was 2.0 years (interquartile range [IQR] 0.5, 6.1), and 67% were male. Thirteen (20.3%) of the final biopsies were obtained from explants. Participants with ALGS had higher levels of conjugated bilirubin ( $P = 0.028$ ), alanine aminotransferase (ALT) ( $P = 0.003$ ), and AST ( $P = 0.034$ ) than those with PFIC. Median APRI was also higher in ALGS (2.1 vs. 1.1;  $P = 0.033$ ). There were no differences in FIB-4 (0.1 vs. 0.1;  $P = 0.934$ ) or platelet levels (310.5 vs. 379.5;  $P = 0.276$ ) between disease groups at time of final biopsy (Supporting Table S2).

When stratified by individual fibrosis stages (F0, F1, F2, F3, or F4) at the final biopsy, there were no differences in median age ( $P = 0.917$ ) or in levels of AST, ALT, or conjugated bilirubin. Platelet levels were lowest in participants with F4 ( $P = 0.027$ ). APRI

scores were highest in participants with F4 ( $P < 0.001$ ; Supporting Table S3 and Fig. S1).

## NONSEVERE (F0-F2) VERSUS SEVERE (F3-F4) FIBROSIS

### F0-F2 Versus F3-F4 in ALGS

Thirteen of 40 (32.5%) participants with ALGS were staged as F3-F4 at final biopsy. Among ALGS, there was no difference in age (years) at time of biopsy between F3-F4 and F0-F2 (3.5 vs. 1.6;  $P = 0.319$ ). There were also no differences in AST, ALT, platelets, conjugated bilirubin, or GPR between those with F3-F4 versus F0-F2. However, ALGS with F3-F4 had higher APRI (3 vs. 1.7;  $P = 0.029$ ) but not FIB-4 (0.3 vs. 0.1;  $P = 0.076$ ) (Table 1).

The AUC of log-transformed APRI and FIB-4 to distinguish F3-F4 in ALGS was 0.72 ( $P = 0.012$ )

and 0.68 ( $P = 0.039$ ), respectively (Fig. 1A). An APRI greater than 2.97 demonstrated a sensitivity (95% confidence interval [CI]) of 61.54% (0.32, 0.86) and specificity of 81.48% (0.62, 0.94) in ALGS. With a 50% increase in APRI, the odds of F3-F4 were 1.31-fold higher (95% CI: 1.04, 1.65;  $P = 0.023$ ) in ALGS. Log-transformed conjugated bilirubin (AUC = 0.69;  $P = 0.121$ ) was unable to distinguish F3-F4 in participants with ALGS (Fig. 1A).

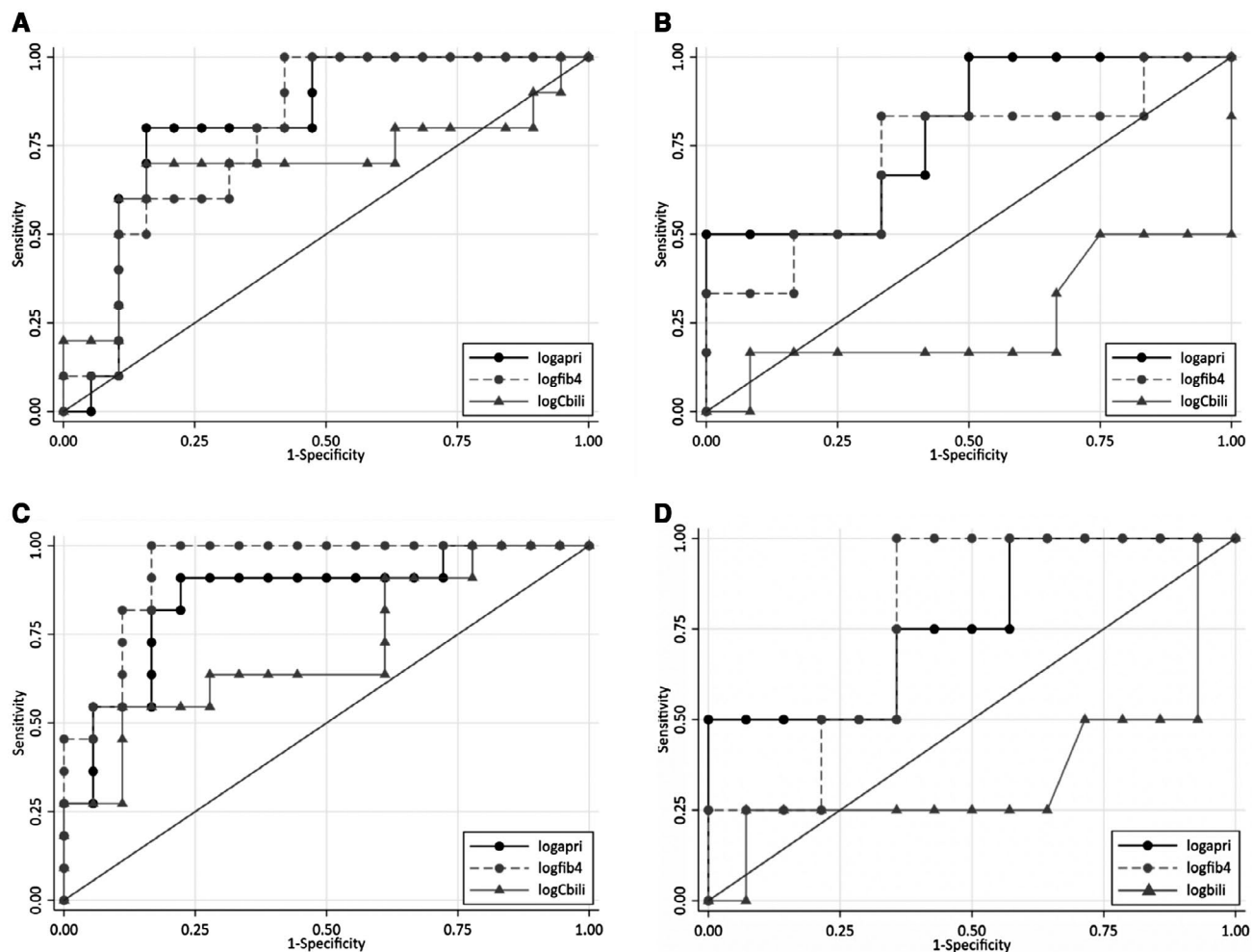
### F0-F2 Versus F3-F4 in PFIC

Ten of 24 (41.7%) participants with PFIC were staged as F3-F4 at final biopsy. Among PFIC, there was no difference in age at time of biopsy (years) between F3-F4 and F0-F2 (2.2 vs. 1.6;  $P = 0.682$ ). There were also no differences in AST, ALT, platelets, or FIB-4 between those with F3-F4 and F0-F2.

TABLE 1. CLINICAL CHARACTERISTICS BY FIBROSIS SEVERITY

Variable	ALGS (n = 40)					PFIC (n = 24)				
	n	F0-F2 (n = 27)	n	F3-F4 (n = 13)	PValue*	n	F0-F2 (n = 14)	n	F3-F4 (n = 10)	PValue*
Fibrosis stage (%)										
		F0		9 (22.5)			F0		7 (29.2)	
		F1		4 (10.0)			F1		3 (12.5)	
		F2		14 (35.0)			F2		4 (16.7)	
		F3		9 (22.5)			F3		5 (20.8)	
		F4		4 (10.0)			F4		5 (20.8)	
Male (%)	27	16 (59.3)	13	11 (84.6)	0.157	14	9 (64.3)	10	7 (70.0)	1.000
Genetic confirmation (%)	27	11 (40.7)	13	7 (53.8)	0.509	14	8 (57.1)	10	7 (70.0)	0.678
Age at biopsy (years)	27	1.6 (0.2, 4.5)	13	3.5 (1.3, 6.4)	0.319	14	1.6 (0.6, 8.4)	10	2.2 (1.4, 3.1)	0.682
		Median (IQR)		Median (IQR)			Median (IQR)		Median (IQR)	
Platelets ( $10^9$ )	27	415 (208, 526)	13	251 (174, 281)	0.067	14	416 (351, 512)	10	333 (153, 454)	0.089
ALT (IU/L)	27	151 (111, 238)	13	203 (126, 215)	0.908	14	69.5 (41, 196)	10	95 (73, 154)	0.482
AST (IU/L)	27	177 (118, 282)	13	294 (155, 326)	0.059	14	103 (77, 132)	10	168.5 (96, 298)	0.266
Conjugated bilirubin (mg/dL)	21	4.8 (3.3, 5.6)	12	7.5 (1.8, 9.3)	0.294	12	3.4 (2.3, 4.8)	9	0.8 (0.0, 2.4)	0.011
GGT (U/L)	26	342 (171, 563)	13	263 (160, 528)	0.623	13	25 (20, 39)	10	54 (26, 264)	0.063
APRI	27	1.7 (1.0, 2.3)	13	3 (1.9, 6.9)	0.029	14	0.8 (0.7, 1.7)	10	2 (1.0, 6.8)	0.053
FIB-4	27	0.1 (0.0, 0.3)	13	0.3 (0.1, 0.5)	0.076	14	0.0 (0.0, 0.3)	10	0.1 (0.0, 0.6)	0.219
GPR	26	2.6 (1.1, 4.0)	13	2.8 (1.4, 5.8)	0.348	13	25 (20, 39)	10	1 (0.1, 2.0)	0.035

\* $P$  values for median comparisons using two-sample Wilcoxon rank-sum (Mann-Whitney) test;  $P$  values calculated with exact testing for categorical variables when possible; otherwise chi-square test.



**FIG. 1.** ROC curves for APRI, FIB-4, and conjugated bilirubin (cBili) to distinguish F3-F4 from F0-F2 among ALGS or PFIC (A and B, respectively) and in the prediction of risk for LT in ALGS or PFIC (C and D, respectively). (A) In ALGS, APRI (AUC 0.72) was better at distinguishing F3-F4 from F0-F2 than FIB-4 (AUC 0.68) or cBili (AUC 0.69). (B) In PFIC, APRI (AUC 0.74) was better at distinguishing F3-F4 from F0-F2 than FIB-4 (AUC 0.65) or cBili (AUC 0.26). (C) In ALGS, APRI (AUC 0.87) and FIB-4 (AUC 0.84) were better at prediction of risk for LT than cBili (AUC 0.71). (D) In PFIC, FIB-4 (AUC 0.71) was better at the prediction of risk for LT than APRI (AUC 0.66) or cBili (AUC 0.35). Solid line with black dots represents ROC curve of log-transformed APRI. Hashed line with gray dots represents ROC curve of log-transformed FIB-4. Solid line with gray triangles represents ROC curve of log-transformed cBili.

However, PFIC with F3-F4 had lower conjugated bilirubin levels (0.8 vs. 3.4;  $P = 0.011$ ) and higher APRI (2.0 vs. 0.8;  $P = 0.053$ ) (Table 1).

The AUC of log-transformed APRI and FIB-4 to distinguish F3-F4 in PFIC was 0.74 ( $P = 0.039$ ) and 0.65 ( $P = 0.218$ ), respectively (Fig. 1B). An APRI greater than 0.99 demonstrated a sensitivity (95% CI) of 80% (0.44, 0.98) and specificity of 64.29% (0.35, 0.87) in PFIC. In PFIC, a 50% increase in APRI was not statistically associated with higher odds of F3-F4. Log-transformed conjugated bilirubin (AUC = 0.26;

$P = 0.115$ ) was also unable to distinguish F3-F4 in participants with PFIC (Fig. 1B).

## APRI AND FIB-4: LONGITUDINAL ANALYSIS OF FIBROSIS IN ALL PARTICIPANTS (ALGS AND PFIC)

Sixteen of the 64 (25%) participants (ALGS and PFIC) had two or more liver biopsies performed during the study period. Repeat biopsies were performed due to concern for worsening cholestasis

or rising transaminases ( $n = 7$ ) or were retrieved at time of LT or other procedures ( $n = 9$ ). Nine participants were progressors (increase of  $\geq 1$  fibrosis stage between biopsies). The remaining 7 participants were nonprogressors. There were no differences in clinical characteristics (median age of biopsy, labs, APRI, or FIB-4) between progressors and nonprogressors. Time elapsed (years) between biopsies was also not different between groups (2.8 vs. 1.5,  $P = 0.368$ ). Progressors demonstrated an increase of APRI (+2.7), FIB-4 (+0.3), and conjugated bilirubin (+1.3) from time between the first biopsy and final biopsy. In nonprogressors, none of the biomarkers (APRI, FIB-4, and conjugated bilirubin) demonstrated any change from the time of initial biopsy to final biopsy (Table 2). Overall, 6 (37.5%) participants demonstrated an increase of two or more metavir stages between biopsies. Time elapsed between biopsies was not different in subjects who progressed only one stage versus those who progressed two or more stages (3.28 vs. 3.35 years,  $P = 0.952$ ).

Longitudinal analysis of biomarker trends was performed in 40 participants (ALGS and PFIC; 62.5%) who had multiple sets of labs available (AST, ALT, platelets, and conjugated bilirubin) before final biopsy (Fig. 2). A linear mixed model demonstrated that in 1 year, there was a 10% increase in APRI (95% CI = 3.75, 16.57;  $P = 0.001$ ) and a 60.2% increase in

FIB-4 (40.79, 82.21;  $P < 0.001$ ). Using logistic regression, participants who exceeded these percentage changes of APRI and FIB-4 in 1 year, increased their odds of F3-F4 by 1.14-fold ( $P < 0.001$ ) and 1.01-fold ( $P = 0.242$ ), respectively.

## LT VERSUS NO LT

### LT Versus No LT in ALGS

Thirteen of 40 (32.5%) participants with ALGS eventually underwent LT at a median age of 3.5 years (2.4, 4.3). Of the participants with ALGS who had LT, 6 (46%) demonstrated F3-F4 fibrosis at final biopsy. The most common LT indications for ALGS were chronic cholestasis ( $n = 13$ , 100%), intractable pruritus ( $n = 10$ , 76.9%), growth failure/malnutrition requiring either supplemental enteral or parenteral nutrition ( $n = 9$ , 69.2%), deforming xanthomas ( $n = 4$ , 30.8%), and pathological fractures ( $n = 3$ , 23.1%). Median APRI (3.9 vs. 1.7,  $P < 0.001$ ) and FIB-4 (0.4 vs. 0.0,  $P < 0.001$ ) for ALGS LT was higher than ALGS no LT (Table 3).

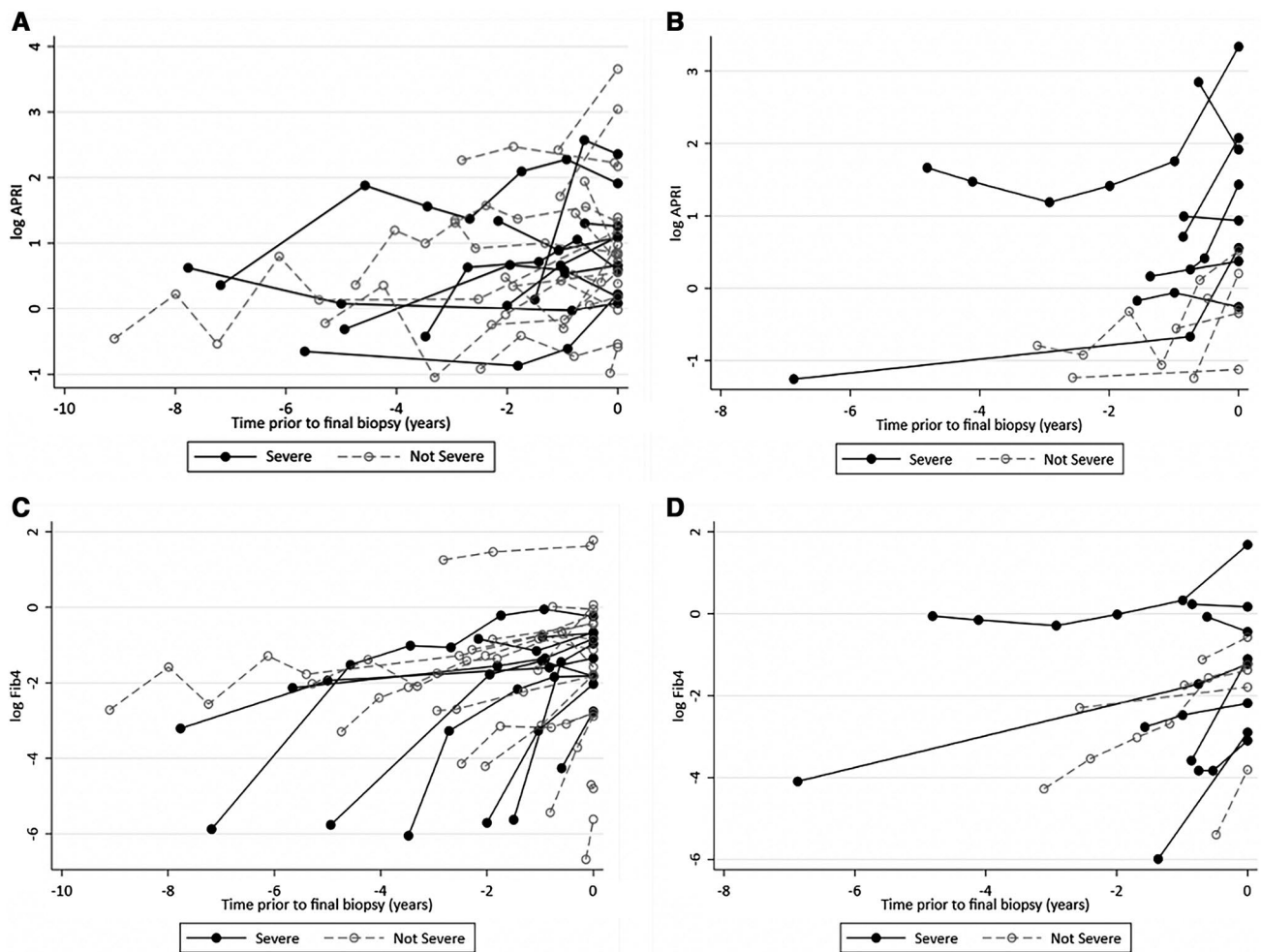
To distinguish participants with ALGS with LT, the AUC of log-transformed APRI, FIB-4, and conjugated bilirubin was 0.87 ( $P < 0.001$ ), 0.84 ( $P < 0.001$ ), and 0.71 ( $P = 0.051$ ), respectively (Fig. 1C). APRI greater than 2.17 had a sensitivity (95% CI) of 92.3% (0.64, 0.99) and specificity of 81.5% (0.62, 0.94). In

TABLE 2. CLINICAL CHARACTERISTICS OF PROGRESSORS VERSUS NONPROGRESSORS

Variable	n	Nonprogressors, n = 7 (ALGS = 6, PFIC = 1)	Progressors, n = 9 (ALGS = 7, PFIC = 2)	PValue*
		Median (IQR)	Median (IQR)	
Age at biopsy (years)	7	2.4 (1.3, 3.4)	1.7 (1.4, 5.0)	0.874
Platelets ( $10^9$ )	7	526 (310.0, 537.0)	279 (194.0, 418.0)	0.125
ALT (IU/L)	7	129 (93.0, 212.0)	174 (126.0, 215.0)	0.427
AST (IU/L)	7	118 (63.0, 301.0)	326 (120.0, 399.0)	0.112
Conjugated bilirubin (mg/dL)	7	2.9 (0.0, 4.8)	6.7 (0.0, 8.5)	0.453
GGT (U/L)	7	175 (83.0, 563.0)	160 (52.0, 528.0)	0.634
APRI	7	0.8 (0.4, 2.7)	3.9 (1.2, 6.9)	0.186
FIB-4	7	0.1 (0.0, 0.3)	0.3 (0.0, 0.4)	0.634
Time between biopsies (years)	7	2.8 (2.0, 6.9)	1.5 (0.8, 4.7)	0.368
$\Delta$ APRI <sup>†</sup>	7	0 (0, 1.7)	+2.7 (0.5, 5.9)	0.082
$\Delta$ FIB-4 <sup>†</sup>	7	0 (0, 0.2)	+0.3 (0, 0.3)	0.050
$\Delta$ Conjugated <sup>†</sup> bilirubin (mg/dL)	5	0 (0, 0)	+1.3 (-0.2, 6.8)	0.651

\*P values for median comparisons using two-sample Wilcoxon rank-sum (Mann-Whitney) test; P values calculated with exact testing for categorical variables when possible; otherwise chi-square test.

<sup>†</sup> $\Delta = (\text{APRI, FIB-4, or cBili at final biopsy}) - (\text{APRI, FIB-4, or cBili at the patient's first biopsy})$ .



**FIG. 2.** Chronological plot of APRI or FIB-4 in patients (with two or more sets of labs available) leading up to the time of final biopsy. There is a visual overall upward trend in biomarkers when followed longitudinally. (A) APRI over time in ALGS. (B) APRI over time in PFIC. (C) FIB-4 over time in ALGS. (D) FIB-4 over time in PFIC. Individual dots represent log-transformed APRI or FIB-4 calculated before the time of final biopsy, which is represented by time point = 0. Filled dots represent APRI or FIB-4 levels of patients who had F3-F4 fibrosis at final biopsy. Open dots represent APRI or FIB-4 levels of patients who had F0-F2 fibrosis at final biopsy.

ALGS, a 50% increase in APRI was associated with 1.97-fold higher odds for LT (95% CI: 1.20, 3.24;  $P = 0.007$ ).

### LT Versus No LT in PFIC

Six of 24 (25%) participants with PFIC eventually underwent LT at a median age of 6.8 years. Of the participants with PFIC who had LT, 5 (83%) demonstrated F3-F4 fibrosis at final biopsy. The most common LT indications for PFIC were intractable pruritus ( $n = 4$ , 66.7%), malnutrition requiring supplemental enteral nutrition ( $n = 3$ , 50%), chronic cholestasis

( $n = 2$ , 33%), and portal hypertension with ascites ( $n = 2$ , 33%). Median APRI (2.0 vs. 0.9,  $P = 0.257$ ) and FIB-4 (0.2 vs. 0.0,  $P = 0.033$ ) for PFIC LT was higher than PFIC with no LT (Table 3).

To distinguish participants with PFIC with LT, the AUC of log-transformed APRI, FIB-4, and conjugated bilirubin was 0.66 ( $P = 0.315$ ), 0.80 ( $P = 0.002$ ), and 0.35 ( $P = 0.473$ ), respectively (Fig. 1D). FIB-4 greater than 0.11 had a sensitivity (95% CI) of 83.3% (0.36, 0.99) and specificity of 66.7% (0.41, 0.87). In PFIC, a 50% increase in FIB-4 was associated with 1.36-fold higher odds for LT (95% CI: 1.28, 1.44;  $P < 0.001$ ).

TABLE 3. CLINICAL CHARACTERISTICS BY LT

Variable	ALGS			PValue*	Variable	PFIC			PValue*
	Yes LT, n = 13 (%)	No LT, n = 27 (%)	Median (IQR)			Yes LT, n = 6 (%)	No LT, n = 18 (%)	Median (IQR)	
Male (%)	8 (61.5)	19 (70.4)		1.000	Male (%)	5 (83.3)	11 (61.1)		0.621
F3-F4 <sup>†</sup>	6 (46.2)	7 (25.9)		0.284	F3-F4 <sup>†</sup>	5 (83.3)	5 (27.8)		0.050
F0-F2 <sup>†</sup>	7 (53.8)	20 (74.1)			F0-F2 <sup>†</sup>	1 (16.7)	13 (72.2)		
LT indications					LT indications				
Chronic cholestasis	13 (100.0)				Intractable pruritus	4 (66.7)			
Intractable pruritus	10 (76.9)				Malnutrition	3 (50.0)			
Malnutrition	9 (69.2)				Chronic cholestasis	2 (33.3)			
Deforming xanthomas	4 (30.8)				Ascites	2 (33.3)			
Pathological fractures	3 (23.1)								
	n	Median (IQR)	n	Median (IQR)	n	Median (IQR)	n	Median (IQR)	
Age at biopsy (years)	13	2.9 (2.1, 4.3)	27	0.9 (0.2, 5.8)	6	6.8 (1.7, 11.7)	18	1.2 (0.6, 3.4)	0.077
Age at LT (years)	13	3.5 (2.4, 4.3)	0		6	6.8 (1.7, 11.7)	0		
Platelets (10 <sup>6</sup> )	13	194 (62, 279)	27	420 (267, 528)	6	262.5 (153, 512)	18	404 (334, 505)	0.230
ALT (IU/L)	13	186 (129, 211)	27	151.0 (114.0, 238.0)	6	114 (42, 154)	18	84.5 (43, 196)	0.815
AST (IU/L)	13	247 (163, 326)	27	205 (101, 294)	6	175 (79, 298)	18	109.5 (80, 216)	0.739
Conjugated bilirubin (mg/dL)	11	8.1 (4.3, 18.2)	22	4.2 (0.8, 5.6)	5	0.8 (0.8, 2.4)	16	2.8 (1.9, 4.0)	0.341
GGT (U/L)	12	254 (143, 422)	27	413.0 (175, 604)	6	51 (20, 345)	17	31.0 (21, 49)	0.344
APRI	13	3.9 (2.7, 10.6)	27	1.7 (1.0, 2.1)	6	2.0 (0.8, 8.0)	18	0.9 (0.7, 2.3)	0.257
FIB-4	13	0.4 (0.2, 1.1)	27	0.0 (0.0, 0.3)	6	0.2 (0.1, 1.2)	18	0.0 (0.0, 0.3)	0.033
GPR	12	4.4 (2.0, 5.5)	27	413 (175, 604)	6	0.5 (0.1, 5.6)	17	0.2 (0.1, 0.4)	0.257

\*P values for median comparisons using two-sample Wilcoxon rank-sum (Mann-Whitney) test; P-values calculated with exact testing for categorical variables when possible; otherwise chi-square test.

<sup>†</sup>Most recent biopsy of native liver or at explant.



## Discussion

The heterogeneity of liver disease in ALGS and PFIC can present prognosticating challenges to pediatric hepatologists. Over the past two decades, efforts have been made to validate biomarker indices such as APRI and FIB-4 in pediatric liver disorders.<sup>(22,23,26-31)</sup> We evaluated the utility of APRI, FIB-4, and conjugated bilirubin as biomarkers of liver fibrosis and as predictors of LT in children with ALGS and PFIC. Importantly, our findings are reported from a very young and cholestatic cohort, with close to 35% of our participants demonstrating greater than F3 fibrosis and 10% with cirrhosis at the time of final biopsy. This suggests the potential for rapid and early progression of liver disease in ALGS and PFIC and emphasizes the importance of affordable and reproducible noninvasive alternatives to liver biopsy for reliable assessment of fibrosis and monitoring of disease progression.

In this study, APRI demonstrated fair discriminative ability to distinguish severe fibrosis (F3-F4) among participants with ALGS, exhibiting high specificity with a cutoff of over 2.97. Similarly, when applied to participants with PFIC, APRI exhibited high sensitivity to distinguish F3-F4 with a cutoff of over 0.99. APRI was not as adept at distinguishing between individual metavir stages, although there was a statistical increase as fibrosis worsened in both disease groups. Furthermore, APRI was two times higher in participants with ALGS and PFIC with F3-F4. These findings support that elevated or rising APRI may be associated with hepatic disease progression in ALGS and PFIC with suggested biopsy-validated cutoffs for clinical prediction of severity of fibrosis. Although clinical decision making in children with ALGS or PFIC is often based on the development of extrahepatic manifestations such as intractable pruritus, disfiguring xanthomas, and severe malnutrition from persistent cholestasis,<sup>(2,4,32)</sup> identification of worsening fibrosis severity in these children remains an important prognostic variable, as advanced stages of fibrosis not only lead to complications from cirrhosis but contribute to and parallel the severity of these extrahepatic features. Therefore, despite a somewhat limited sensitivity, APRI appears to be a readily available, noninvasive tool that may complement the overall decision-making process in children with rare cholestatic disorders.

Multiple pediatric studies, including ours, have demonstrated superior utility of APRI in fibrosis detection when compared with FIB-4 and other various biomarkers. In a previous study of 67 children with CFLD, APRI outperformed FIB-4 in its ability to distinguish F3-F4 versus F0-F2 and was superior in differentiating patients with CFLD versus those without CFLD.<sup>(23)</sup> In a separate investigation of 77 children with NAFLD, APRI was superior to FIB-4 in distinguishing F0-F1 from F2-F3.<sup>(28)</sup> A potential disadvantage of FIB-4 in pediatrics may stem from the inclusion of age, as this variable may lead to a diminished effect when applied to particularly young and narrow age ranges. In our study, we also evaluated conjugated bilirubin as a potential biomarker of severe fibrosis. Our results demonstrated that conjugated bilirubin may not be a reliable biomarker of liver fibrosis severity in PFIC or ALGS, as it was unable to distinguish F3-F4 from F0-F2 among participants in either disease group. Various reports have demonstrated the utility of GPR in distinguishing fibrosis levels in hepatocellular diseases,<sup>(33-35)</sup> and most recently in pediatric CFLD.<sup>(25)</sup> However, there were no differences in GPR when compared between F3-F4 versus F0-F2 in participants with ALGS. Furthermore, GPR is confounded by the low to normal levels of GGT found in PFIC subtypes 1 and 2, as evidenced by lower GPR levels among participants with PFIC with F3-F4 (Table 1).

Understanding the degree of injury and progression of liver fibrosis over time in children with ALGS and PFIC is important in the complex decision making regarding novel medical therapies,<sup>(36)</sup> as well as for the timing of liver transplant evaluation.<sup>(9,10)</sup> Although imaging modalities using elastography have emerged within pediatrics,<sup>(37)</sup> they remain expensive and are not yet widely available in all pediatric institutions. In contrast, APRI and FIB-4 require only standard-of-care laboratories to calculate and can be easily tracked over time. In our study, we uniquely describe the trajectory of APRI and FIB-4 in a longitudinal manner among participants with ALGS and PFIC. Our results suggest that participants with ALGS or PFIC who exceed specific percentage changes in APRI and FIB-4 over a 1-year period are at greater risk for development of F3-F4. Furthermore, our findings demonstrate that fibrosis progressors had clinically more abnormal liver biochemistries and platelet counts than

nonprogressors at time of final biopsy. Although there was no difference in the time elapsed between biopsies, progressors were generally younger and demonstrated clinically notable changes in APRI, FIB-4, and conjugated bilirubin from time of initial to final biopsy. In comparison, nonprogressors did not demonstrate any change in APRI or FIB-4 from biopsy to biopsy. Unfortunately, while these findings are unique, our small subcohort sample size provided insufficient power for statistical significance.

Few pediatric studies have investigated the utility of APRI or FIB-4 as a biomarker of clinical outcome. In a Finnish study of 29 patients with biliary atresia, APRI was evaluated as a predictor of native liver survival when collected at the time of Kasai portoenterostomy (KPE). Although this study showed no correlation of APRI with metavir (or Ishak) fibrosis, APRI at the time of KPE was significantly higher in the 10 patients who eventually underwent LT.<sup>(31)</sup> In our analysis, APRI, FIB-4, and conjugated bilirubin at the time of final biopsy were all significantly higher in the participants who eventually underwent LT versus those who did not. Among participants with ALGS, APRI and FIB-4 demonstrated good prediction of those who eventually underwent LT. While conjugated bilirubin demonstrated fair ability (AUC 0.71) to predict risk for LT in ALGS, statistical significance was not achieved ( $P = 0.051$ ). Among participants with PFIC, only FIB-4 demonstrated good prediction of those who eventually underwent LT. We acknowledge that the processes of listing and prioritization for LT are subjective, and that children with ALGS or PFIC may be transplanted for quality-of-life indications such as medically refractory pruritus and malnutrition, although worsening fibrosis often contributes to and corresponds with progression of these symptoms. As such, the potential use of APRI and FIB-4 as an additional complementary tool may further guide decision making and timing of LT evaluation for children with these rare diseases.

Limitations of this pediatric study include a moderate sample size and generalization of PFIC subtypes 1-3, although strict inclusion standards requiring either genetic confirmation or *a priori* clinical criteria and the validation of metavir fibrosis staging by a single, blinded pediatric-trained hepatopathologist were implemented. Fewer subjects had data available for conjugated bilirubin, longitudinal,

and LT analysis. For study inclusion, all participants were required to have at least one liver biopsy. Hence, our cohort may be skewed toward more severe liver disease, as it is possible that participants with milder disease did not undergo liver biopsy during this study period. However, it should be acknowledged that it is often the stable patient without significant liver dysfunction or coagulopathy who can safely undergo liver biopsy. Furthermore, participants with more than one biopsy were subject to potential bias, as biopsies were retrieved primarily at the time of explant or for concern for worsening liver disease. We did not analyze bile acids as a biomarker, as they were not readily available in all participants. Although 3 participants had labs collected outside the 90-day window (100, 134, and 162 days) for APRI and FIB-4 calculations, we felt their inclusion was important for the overall analysis of these rare orphan disorders. Prospective, large-scale studies involving multicenter cohorts or international database consortiums will be needed in the future for further validation of these findings.

In conclusion, the evaluation of noninvasive alternatives to liver biopsy for fibrosis monitoring continues to be an essential and developing field in pediatric liver disorders. Investigation of our young cohort of cholestatic children appears to validate the use of APRI as a noninvasive biomarker for liver fibrosis severity in both ALGS and PFIC. In addition, APRI or FIB-4, but not conjugated bilirubin, appear to associate with risk for future LT in children with ALGS or PFIC, providing an alternative method of assessment that may complement other existing processes of evaluation for liver transplant candidacy.

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## Supporting Information

Additional Supporting Information may be found at [onlinelibrary.wiley.com/doi/10.1002/hep4.1569/supinfo](https://onlinelibrary.wiley.com/doi/10.1002/hep4.1569/supinfo).