A Combination of the Pediatric NAFLD Fibrosis Index and Enhanced Liver Fibrosis Test Identifies Children With Fibrosis

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See related articles, Conjeevaram HS et al on page 469 and Bajaj JS et al on page 478, in *Gastroenterology*.

BACKGROUND & AIMS: Nonalcoholic fatty liver disease (NAFLD) encompasses diseases from simple steatosis, to steatohepatitis, to fibrosis, and cirrhosis. The pediatric NAFLD fibrosis index (PNFI) and the enhanced liver fibrosis (ELF) test are potential noninvasive markers for fibrosis. We prospectively evaluated the performance of PNFI and ELF in assessing fibrosis in children with biopsy-proven NAFLD. **METHODS:** We analyzed 111 consecutive children with NAFLD. The stage of fibrosis was scored according to the Nonalcoholic Steatohepatitis Clinical Research Network. PNFI was calculated based on age, waist circumference, and levels of triglycerides. The ELF test was used to determine levels of hyaluronic acid, the amino-terminal propeptide of type III collagen, and tissue inhibitor of metalloproteinase-1. **RESULTS:** Some degree of fibrosis was detected in 68.5% of patients (62 had stage 1, 5 had stage 2, and 9 had stage 3). PNFI and ELF test values was higher among patients with fibrosis ($P < .001$). The area under the receiver operating characteristic (ROC) curve for predicting fibrosis using the PNFI and ELF test was 0.761 and 0.924, respectively. The best performance was obtained by combining PNFI and ELF test with (area under the receiver operating characteristic curve = 0.944). The combined results from the PNFI and ELF test predicted the presence or absence of fibrosis in 86.4% of children with NAFLD. **CONCLUSIONS: In children with NAFLD, the combined results from the PNFI and ELF test can accurately assess the presence of liver fibrosis and identify patients that should be evaluated by liver biopsy.**

Keywords: Nonalcoholic Steatohepatitis (NASH); Noninvasive Tests; Diagnostic Algorithm; Histological Severity.

Nonalcoholic fatty liver disease (NAFLD) has become the most common chronic liver disease in children.[1–3] The spectrum of NAFLD ranges from simple steatosis, to nonalcoholic steatohepatitis (NASH), to fibrosis, and eventually cirrhosis and its complications.[4] The prognosis of NAFLD in children is not clearly defined; however, in the largest natural history study in children to date, up to 80% of patients with repeat biopsies developed some degree of fibrosis during the follow-up period.[5] Liver fibrosis is the most worrisome histological feature in patients with NAFLD, and the early identification of fibrosis in children may play a significant role in preventing the development of advanced liver disease.[6] Liver biopsy is currently the gold standard to diagnose fibrosis; however, it is an invasive and costly procedure that is not suitable as a screening test especially in children. Several groups have developed noninvasive panels of tests to predict the stage of liver fibrosis in adult patients with NAFLD. These can be divided into panels that use clinical and routine laboratory tests and panels that require specialized tests such as direct markers of fibrosis.[7–12] We have recently developed the pediatric NAFLD fibrosis index (PNFI) which is obtained from 3 simple measures (age, waist circumference [WC], and triglycerides [TG]) to predict liver fibrosis in children with NAFLD.[13] A value of 9 or higher could be used to rule in fibrosis and a value of less than 3 could rule out fibrosis. The main limitation to using the PNFI is that most patients fall between these 2 cutoff values so the presence or absence of fibrosis cannot be predicted. We have also investigated the performance of the enhanced liver fibrosis (ELF) test in assessing liver fibrosis in pediatric patients.[14] The ELF test uses a combination of 3 extracellular matrix components, namely hyaluronic acid (HA), amino terminal propeptide of type III collagen (PIIINP), and inhibitor of metalloproteinase 1 (TIMP-1). Despite having acceptable accuracy in predicting the different stages of liver fibrosis, this test requires specialized tests which are not readily available and incur extra costs.

The first aim of the present study was to prospectively evaluate the performance of PNFI, ELF, and their combination in assessing fibrosis in children and adolescents with biopsy-proven NAFLD. The second aim was to generate an algorithm that can be used by clinicians to select patients for liver biopsy, avoid unnecessary biopsies, minimize cost, and increase accuracy in predicting fibrosis in this group of patients.

Abbreviations used in this paper: BMI, body mass index; BP, blood pressure; ELF, enhanced liver fibrosis; HA, hyaluronic acid; ln, Logarithm; NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; PIIINP, propeptide of type III collagen; PNFI, pediatric NAFLD fibrosis index; ROC, receiver operating characteristic; TG, triglycerides; TIMP-1, inhibitor of metalloproteinase 1; WC, waist circumference.

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Methods

Patients

A total of 111 consecutive patients diagnosed with NAFLD (73 male and 38 female) seen at Bambino Gesù Children's Hospital from January 2007 to June 2009 were included in the study. The study was approved by the Ethics Committee of the Bambino Gesù Children's Hospital and Research Institute, Rome, Italy.

Inclusion criteria were persistently elevated serum aminotransferase levels, diffusely hyperechogenic liver on ultrasonography suggestive of fatty liver, and biopsy consistent with the diagnosis of NAFLD.[15,16] Exclusion criteria were hepatic virus infections (hepatitis A, B, C, D, E, and G; cytomegalovirus; and Epstein–Barr virus), alcohol consumption, history of parenteral nutrition, and use of drugs known to induce steatosis (eg, valproate, amiodarone, or prednisone) or to affect body weight and carbohydrate metabolism. Autoimmune liver disease, metabolic liver disease, Wilson's disease, celiac disease, and α-1-antitrypsin deficiency were ruled out using standard clinical, laboratory, and histological criteria.

The body mass index (BMI) and its standard deviation score (Z score) were calculated.[17,18] The metabolic syndrome (MS) was defined as the presence of $\geq$3 of the following 5 criteria:[19] abdominal obesity as defined by a waist circumference $\geq$90th percentile for age;[20] hypertriglyceridemia as defined by TG $>$95th percentile for age and sex;[21] low high-density lipoprotein (HDL) cholesterol as defined by $<$5th percentile for age and sex;[21] elevated blood pressure (BP) as defined by systolic or diastolic BP $>$95th percentile for age and sex;[22] and impaired fasting glucose, impaired glucose tolerance (IGT), or known type 2 diabetes mellitus as described in detail elsewhere.[23]

Laboratory Assessment

The homeostasis model assessment index of insulin resistance (HOMA-IR) and the insulin sensitivity index (ISI) were calculated as surrogate markers of insulin sensitivity.[24,25] The PNFI was calculated using age, WC, and TG as described previously.[13]

The simplified ELF algorithm[8] comprises HA, PIIINP, and TIMP-1 combined in the following algorithm:

$$\text{Discriminant score} = -7.412 + [(\ln \text{HA} * 0.681) + (\ln \text{PIIINP} * 0.775) + (\ln \text{TIMP-1} * 0.494)] + 10.$$

HA, PIIINP, and TIMP-1 were assayed using specifically manufactured highly sensitive enzyme-linked immunosorbent assays on an automated IMMUNO 1 immunoanalyzer (Siemens Medical Solutions Diagnostics, Tarrytown, NY).

Liver Histology

The clinical indication for biopsy was either to assess the presence of NASH and degree of fibrosis or other likely independent or competing liver diseases. Liver biopsy was performed in all children, after an overnight fast, using an automatic core biopsy 18 gauge needle (Biopince, Amedic, Sweden) under general anesthesia and ultrasound guidance. A Sonoline Omnia ultrasound machine (Siemens, Munich, Germany) equipped with a 5-MHz probe (5.0 C 50, Siemens) and a biopsy adaptor was employed. Two biopsy passes within different liver segments were performed for each subject. The length of liver specimen (in millimeters) was recorded. Only samples with a length $\geq$15 mm and including at least 5–6 complete portal tracts[26] were considered adequate for the purpose of the study. Biopsies were evaluated by a single hepatopathologist who was blinded to clinical and laboratory data. Biopsies were routinely processed (ie, formalin-fixed and paraffin-embedded) and sections of liver tissue, 5 μm thick, were stained with hematoxylin-eosin, Van Gieson, Periodic acid-Schiff diastase, and Prussian blue stain. Liver biopsy features were graded according to the NAFLD activity scoring (NAS) system proposed by Kleiner et al.[27] Fibrosis was scored as 0 = none; 1 = periportal or perisinusoidal fibrosis; 2 = perisinusoidal and portal/periportal fibrosis; 3 = bridging fibrosis; and 4 = cirrhosis. The liver biopsy samples were then classified as either definitive NASH (unequivocally fulfills previously described criteria for steatohepatitis), borderline diagnosis (some but not all histologic features of steatohepatitis), or simple steatosis (isolated fat deposition in hepatocytes).

Statistical Analysis

Continuous variables are presented as median (25th, 75th percentiles) and categorical variables as numbers and percentages. Wilcoxon rank sum tests for continuous and ordinal factors and Pearson χ^2 for categorical factors were used to assess differences between subjects with and without fibrosis. Linear-by-linear association tests were used to assess associations between fibrosis stage, ELF, and PNFI.

Multivariable logistic regression was used to assess whether addition of any clinical characteristic improved prediction of the presence of any fibrosis. An automated stepwise variable selection on 1000 bootstrap samples was performed, and variables with an inclusion fraction of more than 30% were assessed for inclusion. The areas under the receiver operating characteristic (ROC) curves were estimated and compared using De Long's method. A $P < .05$ was considered statistically significant. SAS version 9.2 software (SAS Institute, Cary, North Carolina) and R version 2.9.1 software (The R Foundation for Statistical Computing, Vienna, Austria) were used for all analyses.

Results

Patient Characteristics

Table 1 presents a description of subjects included in the analysis. Seventy-six of 111 patients (68.5%) had some degree of liver fibrosis (62 had stage 1, 5 had stage 2, and 9 had stage 3). Subjects with fibrosis had higher BMI, WC, total bilirubin, and were more likely to have low high-density lipoprotein, impaired glucose tolerance, and or diabetes and metabolic syndrome ($P < .05$). In addition, subjects with fibrosis also had more advanced histological characteristics (steatosis, inflammation, and ballooning) than those without fibrosis ($P < .001$) as shown in Table 2. The median PNFI was 7.8 and the median ELF was 8.6. ELF and PNFI were significantly increased in subjects with fibrosis (Figure 1, Table 2).

Comparison Between ELF and PNFI for Diagnosing Fibrosis Stage

ELF was significantly better than PNFI at differentiating any fibrosis from no fibrosis (area under receiver operating

Table 1. Demographic and Clinical Characteristics of Subjects

Factor	All (n = 111)	Fibrosis (n = 76)	No fibrosis (n = 35)	*P* value
Sex				.99
Male	73 (65.8)	50 (65.8)	23 (65.7)	
Age (*y*)	10.5 (9.5, 11.4)	10.5 (9.4, 11.8)	10.4 (9.6, 11.1)	.37
Weight (*kg*)	50.6 (12.4)	51.3 (13.1)	49.2 (10.7)	.39
Height (*cm*)	145 (135, 153)	144.3 (135, 152.5)	147 (134, 156)	.35
BMI	24.8 (4.4)	25.4 (4.6)	23.4 (3.7)	.018
BMI percentile	97 (93, 98)	98 (94, 99.5)	95 (90, 98)	.022
WC (*cm*)	84 (79, 93)	90 (80, 94)	80 (77, 82)	<.001
WC percentile	97 (90, 97)	97 (97, 97)	89 (89, 90)	<.001
Systolic BP (*mm Hg*)	109 (100, 121)	109.5 (100, 121.5)	108 (100, 120)	.49
Diastolic BP (*mm Hg*)	70 (61, 73)	70 (60.5, 71.5)	70 (61, 73)	.57
Cholesterol (*mg/dL*)	164 (37.1)	164.9 (35.6)	162.1 (40.6)	.72
Triglycerides (*mg/dL*)	87 (69, 123)	88 (72, 130)	80 (67, 116)	.27
ALT (*U/L*)	67 (45, 89)	69.5 (43.5, 101.5)	66 (55, 78)	.58
AST (*U/L*)	45 (39, 59)	49.5 (39, 62)	44 (37, 56)	.24
GGT (*U/L*)	22 (17, 33)	22 (16.5, 40.5)	20 (17, 24)	.053
Total bilirubin (*U/L*)	0.6 (0.4, 0.7)	0.5 (0.4, 0.6)	0.7 (0.6, 0.8)	<.001
Albumin (*g/dL*)	4.6 (0.4)	4.6 (0.3)	4.6 (0.4)	.8
Alkaline phosphatase (*U/L*)	664.5 (539, 801.5)	635 (501, 762)	724 (598, 850)	.065
White cell count (*cells/mm^3*)	7750 (6670, 9080)	8020 (6820, 9190)	7650 (5910, 9050)	.4
Platelet count (*cells/mm^3*)	316,000 (275,000, 356,000)	320,000 (279,000, 350,000)	305,000 (270,000, 377,000)	.85
PT (*%*)	95 (89, 98)	94.5 (90, 98)	95 (88, 99)	.47
HOMA-IR	2.4 (1.6, 3.6)	2.4 (1.6, 3.7)	2.1 (1.6, 3.4)	.68
Metabolic syndrome				
Obesity	97 (87.4)	67 (88.2)	30 (85.7)	.76
Low HDL cholesterol	74 (66.7)	56 (73.7)	18 (51.4)	.021
Hypertriglyceridemia	85 (76.6)	58 (76.3)	27 (77.1)	.92
Hypertension	43 (38.7)	30 (39.5)	13 (37.1)	.81
IGT/Diabetes	50 (45.1)	41 (54.0)	9 (25.7)	.006
MS	89 (80.2)	66 (86.8)	23 (65.7)	.01

NOTE. Values presented as median (25th, 75th percentiles) or n (%). *P* values correspond to Wilcoxon rank sum tests for continuous and ordinal factors and Pearson χ^2 tests for categorical variables.

ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index (calculated as weight in kg/height in m^2); GGT, γ-glutamyltransferase; HDL, high-density lipoprotein; HOMA-IR, homeostasis model assessment index of insulin resistance; IGT, impaired glucose tolerance; MS, metabolic syndrome; PT, prothrombin time.

characteristics curve of 0.924 vs 0.761; $P = .005$) (Supplementary Figure 1, Table 3). The best performance was obtained by combining PNFI and ELF test with an area under the ROC curve of 0.944 (95% confidence interval, 0.917–0.99) (Table 3). As expected, the performance of PNFI was worse for classifying moderate and advanced fibrosis as this score was developed for prediction of any fibrosis. On the other hand, ELF remained a good predictor of fibrosis ≥2 and fibrosis stage 3 (Supplementary Figure 1, Table 3).

Our data confirm that a PNFI ≥9 can be used to rule in liver fibrosis, with a specificity of 91.4%, and a value <3 can be used to confidently rule out fibrosis with a sensitivity of 93.4%. The previously proposed cutoff point of ELF ≥9.28 does provide excellent specificity (100%) but very poor sensitivity (21%) so it could be used to rule in fibrosis but not to rule it out. Our data suggest that a lower cut point of 8.49 would increase the sensitivity to 76.9% while maintaining a high specificity (97%).

Combination of PNFI and ELF to Predict the Presence of Fibrosis

We evaluated the use of the combination of PNFI and ELF to predict the presence of fibrosis in children with NAFLD (Figure 2). We started by using PNFI, which is obtained from 3 very simple measures that are readily available in clinical practice (age, WC, and TG). PNFI <3.47 can rule out liver fibrosis in 69% of patients (11/16) which was confirmed by liver biopsy. Out of the 5 missed cases of fibrosis, 80% were stage 1 fibrosis (4/5), which means that only 1 patient with clinically significant fibrosis ($F \geq 2$) will be misclassified as having no fibrosis. A PNFI >9 can rule in fibrosis in about 92% of patients (34/37). If the PNFI is between 3.47 and 8.99, then the ELF score can be used to differentiate between patients with or without fibrosis. An ELF <8.49 can rule out fibrosis in 76.9% of patients (20/26) and all the 6 patients who were misclassified had stage 1 fibrosis which means that no patient with clinically significant fibrosis will be missed. An ELF ≥8.49 can rule in fibrosis in 97% (31/32) with only 1 patient without fibrosis who had an ELF >8.49. Overall, the combined use of PNFI and ELF test as a first-line approach could predict the presence or absence of fibrosis in 86.4% of children with NAFLD.

Discussion

The principal findings of this study relate to the validation of both the ELF test and PNFI as noninvasive methods to screen for liver fibrosis in children and adolescents with NAFLD. We found that the combination of ELF and PNFI gave the highest accuracy to detect the presence or absence of fibro-

Table 2. Histological Features and Noninvasive Markers (ELF and PNFI)

Factor	All (n = 111)	Fibrosis (n = 76)	No fibrosis (n = 35)	*P* value
Steatosis				.008
<5%	2 (1.8)	2 (2.6)	0 (0.0)	
5%–33%	35 (31.5)	18 (23.7)	17 (48.6)	
34%–65%	51 (46.0)	35 (46.1)	16 (45.7)	
≥66%	23 (20.7)	21 (27.6)	2 (5.7)	
Inflammation				<.001
None	15 (13.5)	4 (5.3)	11 (31.4)	
<2 under 20×	78 (70.3)	56 (73.7)	22 (62.9)	
2–4 under 20×	17 (15.3)	15 (19.7)	2 (5.7)	
>4 under 20×	1 (0.9)	1 (1.3)	0 (0.0)	
Ballooning (n = 98)				<.001
None	48 (49.0)	21 (32.3)	27 (81.8)	
Few	24 (24.5)	18 (27.7)	6 (18.2)	
Many	26 (26.5)	26 (40.0)	0 (0.0)	
NAFLD				<.001
Steatosis	34 (30.6)	13 (17.1)	21 (60.0)	
Borderline	46 (41.4)	34 (44.7)	12 (34.3)	
NASH	31 (27.9)	29 (38.2)	2 (5.7)	
Noninvasive markers				
ELF	8.6 (8.1, 9.1)	8.9 (8.6, 9.2)	8.0 (7.7, 8.1)	<.001
PNFI	7.8 (5.2, 9.3)	8.7 (7.0, 9.4)	5.4 (3.2, 6.9)	<.001

sis on liver biopsy. Moreover, the present study provides for the first time a simple diagnostic algorithm that can be used by clinicians to select patients for liver biopsy. The algorithm starts by using PNFI, which is a simple panel that uses readily available clinical variables. By using high and low cutoff values, significant negative and positive predictive values can be obtained. For patients with PNFI values that fall between the 2 cutoffs, we propose the use of the ELF test to determine if fibrosis is present.

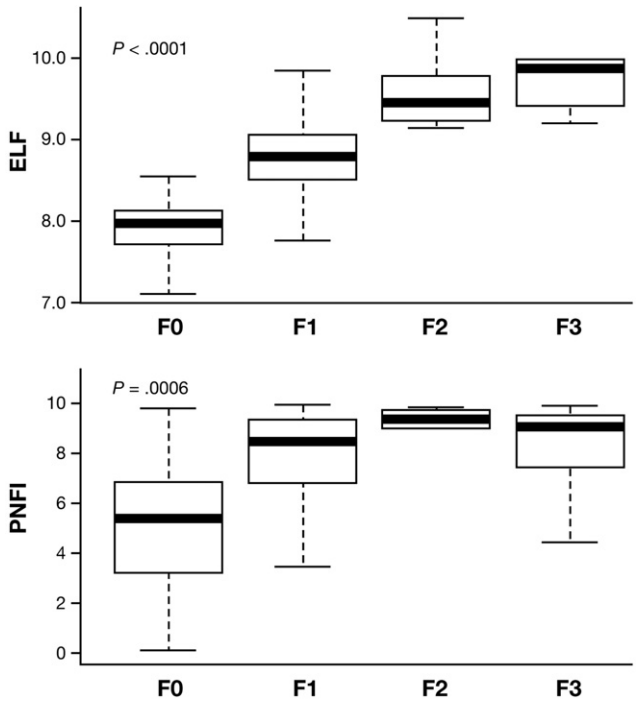

Figure 1. ELF and PNFI for each fibrosis stage. The *lower boundary* of the box-and-whisker plot corresponds to the 25th percentile, the *line within the box* to the median, and the *upper boundary* of the box to the 75th percentile. The whiskers extend to the most extreme data point which is no more than 1.5 times the interquartile range from the box.

NAFLD is the most common chronic liver disease in children and adolescents in industrialized countries and it can progress to cirrhosis and end-stage liver disease during childhood.[5,28–30] The prognosis of NAFLD is heavily dependent on histological severity as determined by liver biopsy. Patients with simple steatosis have a good prognosis, whereas patients with NASH tend to have a progressive disease that can lead to liver-related morbidity and mortality. New evidence suggests that the presence and severity of fibrosis might be the single most important factor in dictating the overall prognosis in NAFLD patients and their risk for progressing to cirrhosis and its complications.[31–33] Although liver biopsy is still considered the accepted standard for staging liver fibrosis, it has several shortcomings including its invasive nature and sampling variability.[34,35] This makes the development of noninvasive tests that can accurately predict the presence of liver fibrosis a high priority especially in the

Table 3. Area Under ROC Curves for Different Fibrosis Thresholds

Model	AUC (95% CI)
Any fibrosis	
ELF + PNFI	0.944 (0.897–0.991)
ELF	0.924 (0.869–0.978)
PNFI	0.761 (0.661–0.861)
Fibrosis 2–3 (clinically significant fibrosis)	
ELF	0.968 (0.937–0.998)
PNFI	0.663 (0.488–0.837)
Fibrosis 3 (advanced fibrosis)	
ELF	0.962 (0.925–0.998)
PNFI	0.618 (0.397–0.839)

AUC, area under receiver operating characteristics curve; CI, confidence interval.

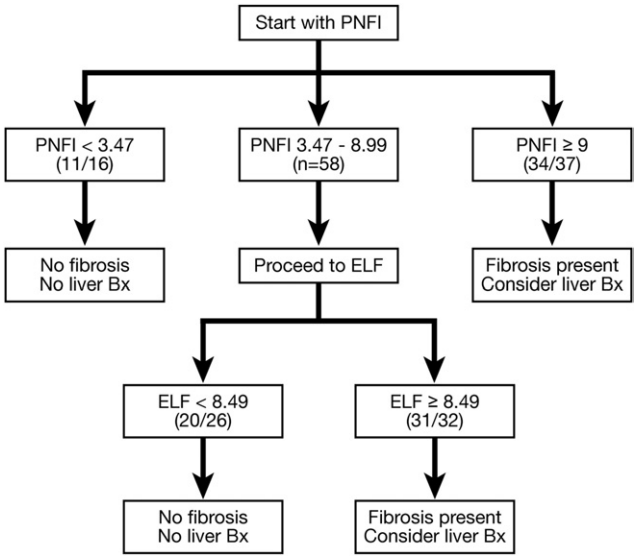

Figure 2. Algorithm using PNFI and ELF to predict the presence or absence of fibrosis in children with NAFLD.

pediatric population where the use of liver biopsy is more controversial.[36]

Three noninvasive approaches have been used for determining the severity of fibrosis in children with NAFLD. The first approach uses a combination of clinical features and routine laboratory tests (age, WC, and TG) to calculate the pediatric NAFLD fibrosis index (PNFI which varies between 0 and 10).[13] This index is easy to calculate with no additional cost to the patient and it has a good positive predictive value to rule in fibrosis; however, its negative predictive value to rule out fibrosis is suboptimal and many patients have values that fall between the 2 suggested cutoff values which makes it impossible to predict the presence or absence of fibrosis. The second approach uses less readily available serum markers of fibrosis including HA, PIIINP, and TIMP-1 to calculate the ELF score. In our original study on using the ELF test in pediatric NAFLD,[14] we found that a cutoff value of 9.28 had a sensitivity of 88% and a specificity of 81% to identify the presence of any fibrosis. The third approach uses radiological methods to detect liver stiffness which can predict the presence and severity of hepatic fibrosis. One such method that has been used in children with fatty liver is transient elastography (TE) which is based on ultrasound technology. We found that a cutoff value of 5.1 kPa had an excellent sensitivity and specificity (97% and 91%, respectively) for the diagnosis of any fibrosis with an area under the receiver operating characteristic curve of 0.977.[37] Transient elastography is not available for clinical use in the United States and its failure rate is reported to be higher in obese individuals which may limit its applicability in NAFLD.[38]

The current study provided evidence that the combination of PNFI and ELF was superior to either test alone in predicting the presence of any fibrosis on liver biopsy. The previously proposed cutoff value of ELF ≥9.28 was found to have a very low sensitivity (21%) for detecting any fibrosis and a new value of 8.49 was found to increase the sensitivity to around 77% without compromising specificity. We developed a diagnostic algorithm that starts by calculating PNFI and then proceeds to ELF if necessary and were able to correctly predict the presence or absence of fibrosis in 86.4% of our cohort as shown in Figure 2. Furthermore, only 1 child with clinically significant fibrosis ($F \geq 2$) was misclassified as having no fibrosis in our entire cohort of 111 patients.

The main strengths of our study are the inclusion of a large group of consecutively recruited children with liver biopsy-proven NAFLD with the full spectrum of disease and different stages of fibrosis. However, our study has some limitations including the fact that patients were seen at a large referral tertiary care medical center and had a high prevalence rate of fibrosis. These results may not apply to children with NAFLD from the community. Another limitation was that most of our children had mild to moderate fibrosis (F1–F2) with only 9 patients with advanced fibrosis (F3) and none with cirrhosis. However, these findings are typical for children with NAFLD and our results resemble those seen in other pediatric series. A large multicenter clinicopathological study by Carter–Kent et al found that 76% of their pediatric population with fibrosis had stage 1 or 2, 24% had stage 3, and none had cirrhosis.[39]

It is important to note that neither PNFI nor the ELF test can distinguish between simple steatosis and NASH and their use should be restricted to children with suspected NAFLD to determine the likelihood of having fibrosis on liver biopsy.

In conclusion, our results support the use of the combination of PNFI and ELF test to accurately assess the presence of liver fibrosis and to identify patients in whom liver biopsy is correctly indicated. Future studies are needed to externally cross-validate our findings before the combination of PNFI and ELF can be recommended in children with NAFLD. Moreover, longitudinal studies measuring these panels serially against clinical outcomes will determine if they can be used to measure disease progression and regression.

Supplementary Material

Note: To access the supplementary material accompanying this article, visit the online version of *Clinical Gastroenterology and Hepatology* at www.cghjournal.org, and at doi:10.1016/j.cgh.2010.09.015.

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Conflicts of interest

The author discloses the following: WMR is a shareholder in iQur Ltd. The remaining authors disclose no conflicts.

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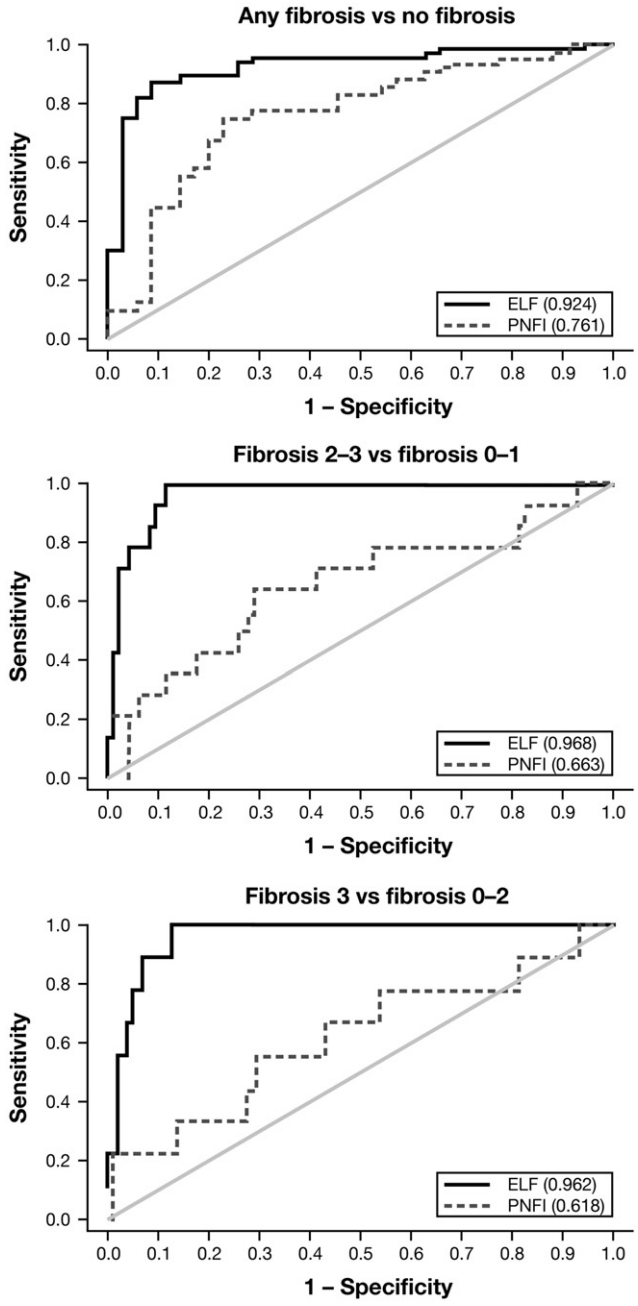

Supplementary Figure 1. ROC curves for ELF and PNFI.