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