Diagnosis of Nonalcoholic Fatty Liver Disease in Children and Adolescents: Position Paper of the ESPGHAN Hepatology Committee

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ABSTRACT

Nonalcoholic fatty liver disease (NAFLD) is the most common cause of chronic liver disease in children and adolescents in the United States, and most probably also in the rest of the industrialized world. As the prevalence of NAFLD in childhood increases with the worldwide obesity epidemic, there is an urgent need for diagnostic standards that can be commonly used by pediatricians and hepatologists. To this end, we performed a PubMed search of the adult and pediatric literature on NAFLD diagnosis through May 2011 using Topics and/or relevant Authors as search words. According to the present literature, NAFLD is suspected based on the association of fatty liver combined with risk factors (mainly obesity), after the exclusion of other causes of liver disease. The reference but imperfect standard for confirming NAFLD is liver histology. The following surrogate markers are presently used to estimate degree of steatosis and liver fibrosis and risk of progression to end-stage liver disease: imaging by ultrasonography or magnetic resonance imaging, liver function tests, and serum markers of liver fibrosis. NAFLD should be suspected in all of the overweight or obese children and adolescents older than 3 years with increased waist circumference especially if there is a NAFLD history in relatives. The typical presentation, however, is in children ages 10 years and older. The first diagnostic step in these children should be abdominal ultrasound and liver function tests, followed by exclusion of other liver diseases. Overweight/obese children with normal ultrasonographic imaging and normal liver function tests should still be monitored due to the poor sensitivity of these tests at a single assessment. Indications for liver biopsy include the following: to rule out other treatable diseases, in cases of clinically suspected advanced liver disease, before pharmacological/surgical treatment, and as part of a structured intervention protocol or clinical research trial.

Key Words: children, histology, imaging, liver biopsy, nonalcoholic fatty liver disease, nonalcoholic steatohepatitis, noninvasive biomarkers, obesity-related liver disease

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Obesity is a major public health concern. The rise in the incidence of obesity diffusion is paralleled by that of its comorbidities, including nonalcoholic fatty liver disease (NAFLD) (1). The latter includes a spectrum of clinicopathological entities ranging from simple steatosis through nonalcoholic steatohepatitis (NASH) to cirrhosis and end-stage liver disease (Table 1). The nomenclature is inconsistent, with NAFLD being both the summarizing term for the entire spectrum of the condition and the descriptor of the more benign forms of simple steatosis and mild inflammation in contrast to NASH. The histopathological definition of steatohepatitis requires at least 5% of liver cells with micro- or macrovesicular fatty infiltration. NAFLD has become the most common chronic hepatopathy both in adults and children. Its histologically proven prevalence in children in the United States (as revealed at autopsy after accidents) ranges from 9.6% in normal-weight individuals up to 38% in obese ones (2). Due to its tendency to progress through this spectrum in childhood (3) or after transition into adulthood (4), early diagnosis and treatment are important issues at all ages (5). Treatment should address not only the liver disease itself but also the entire spectrum of comorbidities to improve overall survival and quality of life (6).

Available diagnostic procedures include a set of clinical signs and symptoms, laboratory and radiological imaging tests, and a combination of clinical parameters and blood test results (7,8). Although several of these markers are commonly used for the diagnostic evaluation of a patient with suspected NAFLD, none of them seems to have a high specificity and sensitivity capable of definitely excluding another underlying liver disease. With the rising prevalence of childhood obesity, the proportion of children with both an underlying primary liver disease, such as autoimmune liver disease or Wilson disease, and additional NAFLD increases, so it becomes essential not to miss a treatable condition. Also, only liver histology can distinguish simple steatosis or mild inflammatory changes from NASH and determine the presence and stage

TABLE 1. Definitions of the spectrum of clinicopathological entities of nonalcoholic fatty liver

Simple steatosis	At least 5% of liver cells with micro- or macrovesicular fatty infiltration
NAFLD	The more benign form of simple steatosis and mild inflammation or The summarizing term for the entire spectrum of the condition
NASH	''Adult type'': steatosis with ballooning degeneration and lobular inflammation, with or without perisinusoidal fibrosis, and without portal inflammation ''Pediatric type'': macrovesicular hepatocellular steatosis with portal inflammation, with or without portal fibrosis, in the absence of ballooning degeneration and perisinusoidal fibrosis
Cirrhosis	The most advanced stage of fibrosis (stage 3 = bridging fibrosis, stage 4 = cirrhosis)

NAFLD = nonalcoholic fatty liver disease; NASH = nonalcoholic steatohepatitis.

of fibrosis (9). At present, liver biopsy remains the ''imperfect'' reference standard for NAFLD diagnosis (10), but it represents an impractical screening procedure because it is both expensive and invasive.

METHODS

We performed a PubMed search by Topics and/or relevant Authors up to May 2011 of the adult and pediatric literature on NAFLD diagnosis using the following terms: "fatty liver, NAFLD, NASH, obesity-related liver disease, liver steatosis" with "diagnosis, genetics, obesity, imaging, liver biopsy, histology, noninvasive biomarkers, liver tests." We selected articles examining conventional and novel diagnostic options in both adults and children. When pediatric data were not available, adult studies were reviewed. The data were written and reviewed by panelists of the European Society for Pediatric Gastroenterology, Hepatology, and Nutrition NAFLD working group and by the members of the European Society for Pediatric Gastroenterology, Hepatology, and Nutrition Hepatology Committee.

GENETIC FACTORS

NAFLD is considered a multifactorial disease with a substantial genetic component. Several studies have shown that some single-nucleotide polymorphisms of genes involved in insulin sensitivity, lipid metabolism, and inflammation/fibrosis may influence both the mechanism and the extent of hepatic steatosis and its progression to NASH and cirrhosis (11). A nonsynonymous single-nucleotide polymorphism (rs738409) in the gene *PNPLA3* (the gene for adiponutrin, an insulin-regulated phospholipase) is associated with hepatic steatosis but not with insulin sensitivity or inflammatory changes at histology in adult and pediatric populations. The 148-mol/L variant has been reported to be associated early in life with increased levels of alanine transaminase (ALT)/aspartate aminotransferase (AST) in a cohort of obese children, with liver steatosis more prevalent in carriers of two 148-mol/L alleles (12–14).

Polymorphisms of interleukin-6 (174G/C) (15) and tumor necrosis factor (TNF)-α (16), both involved in inflammation and insulin resistance, have been associated with NASH. A splice mutation in the tumor suppressor gene Kruppel-like factor 6 (*KLF 6*) has been identified in patients with NAFLD with liver fibrosis (17). It has been shown that variants in the *UGT1A1* gene (Gilbert syndrome) contribute to increased bilirubin levels, thus reducing the risk for NAFLD onset or development (18).

Executive Summary

A combination of genetic and environmental factors is likely responsible for both the development of NAFLD and its progression from simple steatosis to NASH. Several genes involved in lipogenesis and inflammation have been found to have significantly altered expression levels in adults and children with NAFLD, and some polymorphisms in regulatory cascades may be pathogenic. Presently, genetic factors are not relevant in the clinical approach to children with NAFLD.

RISK FACTORS AND CLINICAL AND LABORATORY FEATURES

NAFLD is a diagnosis of exclusion requiring careful consideration of demographic, anthropometric, clinical, and laboratory features. Table 2 illustrates the large spectrum of causes of fatty liver in children (7,19,20).

Risk Factors

NAFLD prevalence is higher in overweight (sex- and age-specific body mass index [BMI] >85th percentile) or obese (>95th percentile) peripubertal male children compared with normal-weight age-matched pairs, and higher in male compared with female age-matched individuals of the same BMI (2,21). Although studies support the association between obesity and NAFLD, rates of obesity and overweight among children with a clinical diagnosis of NAFLD remain variable: Hispanic origin is a risk factor (2,21,22), whereas black race seems to be protective (23).

Familial clustering of obesity, insulin resistance, NAFLD, or type 2 diabetes mellitus is frequent and should raise suspicion of NAFLD in children from such families (24,25). The prevalence of NAFLD is higher in children older than 10 years than it is in younger ones (2), although they do not seem more prone to NASH/fibrosis (22). Unlike in NAFLD, male sex is not a risk factor for NASH (26). Rapid weight increase may be a risk factor for NAFLD in obese children (27).

Low birth weight combined with early catch-up growth is associated with early obesity and is a risk factor for NAFLD (28), whereas breast-feeding seems to reduce the risk (29). Consumption of (rich in fructose) soft drinks seems to be associated with NAFLD, independent of the metabolic syndrome (30). Obstructive sleep apnea (OSA) is associated with insulin-resistant NAFLD, but it does not seem to be related to the severity of steatohepatitis (NASH) (31).

TABLE 2. Causes of fatty liver disease in children

General or systemic	Genetic-metabolic causes	Other rare hereditary genetic disorders	Drugs' hepatotoxicity
Acute systemic disease	Cystic fibrosis and Shwachman syndrome	Alström syndrome	Ethanol
Acute starvation	Wilson disease	Bardet-Biedl syndrome	Ecstasy, cocaine
Protein energy malnutrition	α_1-Antitrypsin deficiency	Prader-Willi syndrome	Nifedipine
Total parenteral nutrition	Galactosemia	Cohen syndrome	Diltiazem
Obesity/metabolic syndrome	Fructosemia	Cantu syndrome (1p36 deletion)	Estrogens
Polycystic ovary syndrome	Cholesteryl ester storage disease	Weber-Christian disease	Corticosteroids
Obstructive sleep apnea	Glycogen storage disease (types I and VI)		Amiodarone
Rapid weight loss	Mitochondrial and peroxisomal defects of fatty acid oxidation		Perhexiline
Anorexia nervosa	Madelung lipomatosis		Coralgil
Cachexia	Lipodystrophies		Tamoxifen
Inflammatory bowel disease	Dorfman-Chanarin syndrome		Methotrexate
Celiac disease	Abeta or hypobetalipoproteinemia		Prednisolone
Hepatitis C	α- and β-oxidation defects		Valproate
Nephrotic syndrome	Porphyria cutanea tarda		Vitamin
Type 1 diabetes mellitus and Mauriac syndrome	Homocystinuria		L-asparaginase
Thyroid disorders	Familial hyperlipoproteinemias		Zidovudine and HIV treatments
Hypothalamo-pituitary disorders	Tyrosinemia type 1		Solvents
Blind loop (bacterial overgrowth)	Bile acids synthesis defects		Pesticides
	Congenital disorders of glycosylation		
	Turner syndrome		
	Organic acidosis		
	Citrin deficiency		
	HFE (hemochromatosis)		

Modified from (7,19,20). Exclusions should be adjusted to age and clinical presentation. In infants and young children, NAFLD is hardly to be expected, whereas genetic, metabolic, syndromic, and systemic causes should be primarily considered guided by clinical signs and symptoms. In children older than 10 years, NAFLD is expected when ≥1 features of the metabolic syndrome are present; still, Wilson disease and α_1-antitrypsin deficiency should be excluded and autoimmune hepatitis should be considered.

Clinical Features

Clinically, most pediatric patients with NAFLD/NASH have nonspecific symptoms. Some complain of fatigue, malaise, or vague abdominal pain (42%–59% of cases), especially in the right upper quadrant, which has been associated with the more progressive form of NASH (2). Acanthosis nigricans is a clinical marker of hyperinsulinemia and has been observed in one-third to half of the children with biopsy-proven NAFLD (23). Hepatomegaly can be frequently detected (up to 50% of cases) (21,22).

Anthropometric Features

Visceral adiposity, which may be related to a state of insulin resistance, is a major contributor to fatty liver, representing a more influential component than BMI in predicting liver steatosis. Unfortunately, indirect measurements of visceral adiposity used in adult studies such as waist-to-hip ratio are not appropriate for childhood because they change with age and have poor correlation with measures of adiposity measured by DEXA (32,33). In children, waist circumference alone represents a practical anthropometric parameter to identify central adiposity and it may predict increased

risk for insulin resistance and the metabolic syndrome (34). Specific percentiles have been developed for children ages 5 to 16 years (35) and 11 to 18 years (36). The importance of waist circumference measurement in childhood NAFLD is well established (37). Lin et al (37) showed that in obese children and adolescents, for every 5-cm increase in waist circumference, there was an odds ratio of 1.4 for predicting ultrasonographic liver steatosis, but no details on percentiles were given. Increased waist circumference is also associated with increased hepatic fibrosis (38). There is a need for standard international waist circumference charts.

Laboratory Tests

In clinical practice the diagnosis of NAFLD is usually suggested by finding elevated serum hepatobiliary enzymes (mostly ALT and γ-glutamyl transpeptidase [GGT]), and/or evidence of a bright liver on ultrasound (US), most frequently among overweight/obese children (27,39,40).

Serum ALT activity is a widely available and inexpensive test for the screening and initial evaluation of NAFLD. The sensitivity of this biochemical marker, however, remains low because a number of adult and pediatric patients may present ALT values in the normal

range (41). In fact, determining an ALT cutoff for NAFLD has been the subject of some debate. In a study involving 72 obese children with NAFLD, an ALT >35 IU/L had a sensitivity of 48% and specificity of 94% for detecting steatosis >5% as measured by magnetic resonance imaging (MRI) (42). More recently, the Screening ALT for Elevation in Today's Youth (SAFETY) study has shown that in American laboratories conventional ALT cutoff values are set too high for the reliable detection of pediatric chronic liver disease, including NAFLD. In the National Health and Nutrition Examination Survey (NHANES) study, the 95th percentile levels for ALT in healthy weight, metabolically normal, liver disease–free patients were 25.8 U/L (boys) and 22.1 U/L (girls) (43). Comparable conclusions were also reached in the European pediatric population (44).

It is now widely accepted that the degree of ALT elevation does not correlate with the presence (42,45) or severity of histological findings of NAFLD (40). A number of children with normal ALT or minimal serum ALT elevation may have advanced fibrosis on liver biopsy. The natural history of the disease is not yet well determined in children, but at times ALT tends to fluctuate and may even normalize (22,41). High serum levels of GGT represent a risk factor for advanced fibrosis in NAFLD (46).

NAFLD may be considered the hepatic manifestation of the metabolic syndrome, which is defined by the presence of visceral obesity, hypertension, insulin resistance or diabetes, dyslipidemia, and hyperuricemia. Hyperinsulinemia, due to insulin resistance, most probably represents the first pathogenetic hit of NAFLD (47). It is a sensitive but nonspecific predictor of NAFLD (48), and hence unsuitable as a single indicator of NAFLD; however, it may be a predictor for progressive hepatic fibrosis (21,26). Abnormalities in the oral glucose tolerance test also may suggest NAFLD (49).

Hypertriglyceridemia is another biochemical marker frequently reported in obese children with NAFLD (21). Oliveira et al (50) showed a positive correlation between ALT and triglyceride values among pediatric patients. Others showed that among patients with suspected steatohepatitis, ALT concentration was significantly higher in subjects with elevated triglycerides (51). Finally, in children with NAFLD, an atherogenic lipid profile correlates with severity of liver injury (52). High levels of serum uric acid have been reported in the majority of subjects with the metabolic syndrome, and has been proposed as an independent predictor of NAFLD both in adults (53) and children (49), probably as a marker of high fructose consumption that correlates with the progression of fibrotic changes (30). Serum IgA level is elevated in about 25% of cases of NAFLD-NASH, but its meaning and diagnostic value are still not clear. High levels of IgA antibodies against tissue transglutaminase have been reported in several chronic liver diseases, including NAFLD (54). Silent celiac disease and fatty liver have been reported to coexist in unrecognized obese children (55).

Increased titers of serum nonorgan-specific autoantibodies (particularly anti-nuclear antibody and anti-smooth muscle antibody) have been reported in up to one-third of all of the investigated patients, both in adults (56) and children (26), and may require immediate definitive further investigation to rule out associated autoimmune hepatitis. Table 3 summarizes the biochemical markers routinely performed in clinical practice in suspected pediatric NAFLD (7,8).

Executive Summary

The panel agreed that careful consideration of a series of anthropometric, demographic, clinical, and laboratory features may offer a clue to the identification of NAFLD risk. Acanthosis nigricans and increased waist circumference are warning signs for NAFLD. ALT in combination with liver ultrasound is an indicator of NAFLD, but normal ALT does not exclude liver steatosis or its progression to severe fibrosis and cirrhosis. Insulin resistance and increased triglyceride concentration are additional risk factors of NAFLD. These factors were identified based on observational studies that associated NAFLD with clinical, anthropometric, and laboratory parameters.

DIFFERENTIAL DIAGNOSIS

Abnormal serum aminotransferases in overweight or obese patients are not diagnostic of NAFLD/NASH. Other causes of muscle (57) and treatable liver disease should be ruled out, with special emphasis on celiac disease–related hepatopathy (55),

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TABLE 3. Laboratory workup in children with suspected NAFLD

Metabolic function and liver tests
    Basic profile: blood counts, standard liver function tests, fasting glucose and insulin, urea and electrolytes, coagulation, INR, ALT/AST ratio
    Lipid profile (cholesterol, triglycerides, HDL-cholesterol, LDL-cholesterol), lipoproteins
    Glucose tolerance test (OGTT), glycosylated hemoglobin
    Calculation of HOMA-IR, ISI-gly as markers for insulin resistance
    Thyroid function tests
Tests for exclusion of other main causes of hepatic steatosis
    Serum lactate, uric acid, iron, ferritin, pyruvate
    Serum copper, ceruloplasmin levels, 24-hour urinary copper
    Sweat test
    Antibodies against tissue transglutaminase IgA and total IgA
    α_1-Antitrypsin levels and phenotype when indicated
    Amino and organic acids
    Plasma-free fatty acids and acyl carnitine profile
    Urinary steroid metabolites
    Other specific tests as suggested by history and examination (eg, viral hepatitis panel, serum immunoglobulins, liver autoantibodies)

Modified from (7,8). ALT = alanine aminotransferase; AST = aspartate aminotransferase; HDL = high-density lipoprotein; HOMA-IR = homeostatic model assessment; INR = international normalized ratio; ISI-gly = insulin sensitivity index; LDL = low-density lipoprotein; OGTT = oral glucose tolerance test.

Wilson disease (58), and autoimmune hepatitis (56,26). ALT serum levels alone are a useful tool, but they are not adequate as a single marker for diagnosing NAFLD. The presence of hepatomegaly or splenomegaly is suggestive of advanced liver disease, which calls for a rapid and complete assessment, including early liver biopsy to exclude other etiologies (19).

The differential diagnosis of NAFLD/NASH is detailed in Table 2 and the proposed workup is outlined in Table 3. NAFLD usually does not occur in extremely young children (younger than 3 years) and is rare in children younger than 10 years. Differential diagnosis should be based first on clinical features, then on blood tests, and finally liver biopsy must be considered (Fig. 1).

Executive Summary

The panel indicates a high suspicion of metabolic disorders as cause of fatty liver in young children. NAFLD hardly occurs in children younger than 3 years and is rare in children younger than 10 years. Thus, young children require a detailed diagnostic work-up to exclude other etiologies. In older children and teenagers, some metabolic disorders should also be considered for differential diagnosis. Obesity per se does not justify making the diagnosis of NAFLD in patients with increased transaminase activity.

THE REFERENCE STANDARD: LIVER BIOPSY

Liver biopsy is the test with the highest discrimination for excluding other potentially treatable conditions. It is also the only single test that can reliably distinguish between simple steatosis (NAFLD) and NASH. As summarized in Table 1, it provides important information regarding the degree of liver damage, changes in the liver architecture, and severity of inflammatory activity and fibrosis (59). Normal liver function tests do not exclude any degree of NAFLD-related liver injury (41). Furthermore, evaluation of liver biopsy may be essential for detecting coexisting diseases (eg, for diagnosis of autoimmune hepatitis).

The principal histological features of NASH include the presence of macrovesicular fatty changes of hepatocytes with displacement of the nucleus to the edge of the cell, ballooning degeneration of hepatocytes, and a mixed lobular inflammation. Other features, such as perisinusoidal-pericellular fibrosis, Mallory hyaline, megamitochondria, acidophil bodies, and glycogenated nuclei, can be present but are not mandatory to establish the diagnosis of NASH (60).

In an effort to standardize the histological criteria, the National Institute of Diabetes and Digestive and Kidney Disease sponsored the NASH Clinical Research Network to develop the NAFLD activity score (NAS) (59). This score is based on the classification proposed earlier by Brunt et al (61) and consists of an unweighted sum for each of the following lesions: steatosis (0–3), lobular inflammation (0–3), and hepatocellular ballooning (0–2). A score ≥ 5 is strongly suggestive of NASH, whereas a score <3 is largely consistent with the absence of NASH; however, the NAS cannot replace a pathologist's diagnosis of steatohepatitis. Furthermore, its utility in assessing the response to therapeutic intervention remains to be determined.
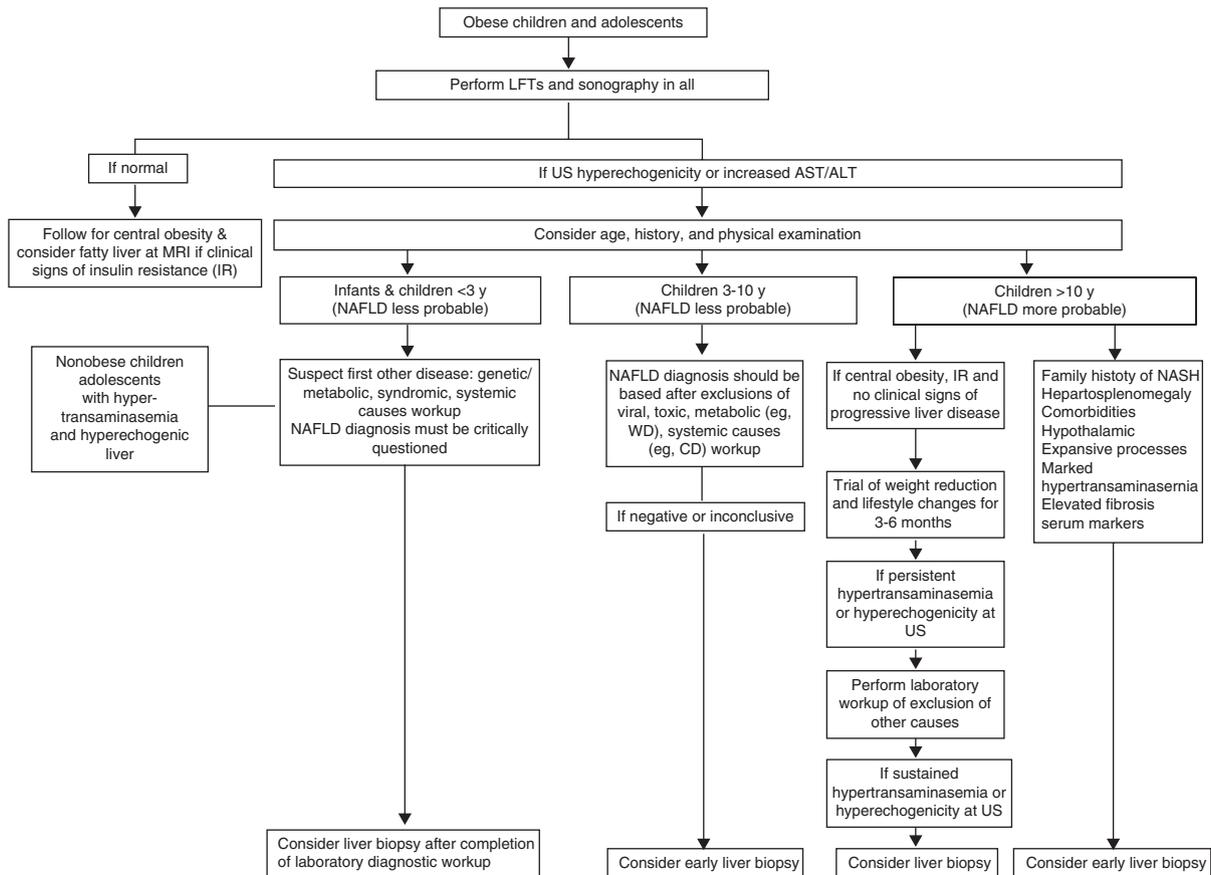
FIGURE 1. Overall management algorithm for children with suspected nonalcoholic fatty liver disease (NAFLD). CD = celiac disease; LFTs = liver function tests; MRI = magnetic resonance imaging; US = ultrasound; WD = Wilson disease.

TABLE 4. Features of noninvasive imaging diagnostic methods in adults with nonalcoholic fatty liver disease

	US	CT	MRI	MRS	MRS elastography (103)	Fibroscan*
Assessment	Qualitative	Qualitative and quantitative	Qualitative and quantitative	Qualitative and quantitative	Qualitative and quantitative	Qualitative and quantitative
Cost	Low	Medium	Expensive	Expensive	Expensive	Low
Method	Visual comparison	Liver attenuation, liver-spleen attenuation ratio	T1 weighted gradient-echo in/out of phase sequence, T2-fat saturated	Spectroscopy, measurement of area under the lipid resonance peak	Modified phase-contrast MRI sequence to visualize propagating share waves in tissues	US transducer probe that measure liver stiffness trough propagation of elastic share wave
Accuracy	Sensitivity: 60%-96%[70,71]; Specificity: 84%-100%[70,71]; Limits: operator dependent	Sensitivity: 82%[77]; Specificity: 100%[77]; Limits: ionizing radiation	Sensitivity: 100%[80]; Specificity: 90.4%[80]; PPV: 50%[81]; NPV: 100%[81]; Overall accuracy 91.2%[81]	Sensitivity: 87%-100%[83]; Diagnostic precision: 80%-85%[83]	Sensitivity: NE; Specificity: NE	Sensitivity: 81%-85%[89]; Specificity: 74%-78%[89]; Limits: fibrosis confused with steatosis if BMI >28
Acquisition time	Variable, 5-20 min, depends on operator	<5 min	10-15 min	10-15 min	10-15 min	<5 min
Availability	Wide in most hospitals	Wide in most hospitals	Software not available in all MRI units	Software not available in all MRI units	Software not available in all MRI units	Not widespread in most hospitals
Threshold of detection	Patients with >20% steatosis	Patients with >30% steatosis	Detect grade I steatosis (5%-30%)	Detect grade I steatosis (5%-30%)	Detect grade I steatosis (5%-30%)	Fibrosis
Distinguishes NASH from NAFLD	No	No	No	No	No	No

Modified from (69). CT = computed tomography; MRI = magnetic resonance imaging; MRS = magnetic resonance spectroscopy; NAFLD = nonalcoholic fatty liver disease; NASH = nonalcoholic steatohepatitis; NE = not evaluated; US = ultrasonography.
*Fibroscan mean values of sensitivity and specificity in pediatric NAFLD were 97%-100% and 91%-100%, respectively. Most accurate values were obtained in cases with higher fibrosis (70).

The histopathological features of NASH in children differ from those found in adults. In a study by Schwimmer et al (22), 100 consecutive liver biopsies of children with NASH, mostly overweight or obese, were analyzed. Based on their analysis, the authors suggested 2 distinct pathological subtypes. Type 1 resembling the adult pattern, characterized by steatosis with ballooning degeneration and lobular inflammation, with or without perisinusoidal fibrosis and without portal inflammation, was seen in 17% of cases. Type 2 was the predominant pattern described in 51% of children, and was defined by macrovesicular hepatocellular steatosis with portal inflammation, with or without portal fibrosis, in the absence of ballooning degeneration and perisinusoidal fibrosis. Studies from Europe (62) and North America (63) have verified that the minority of children have the adult (type 1) pattern of NAFLD. In both of these studies, biopsy findings were more often in an ''overlap'' category (51% and 82%, respectively) than in the type 2 category (60). It is presently unknown what underlies the different histological patterns and whether they represent the hallmark of differences in natural history, etiopathogenesis, prognosis, or response to treatment (22,60).

Considering that percutaneous liver biopsy samples only 1:50,000th of the liver, sampling error is an obvious limitation, which can lead to misdiagnosis and staging inaccuracies. In a study of 51 adult patients with NAFLD, 2 consecutive liver biopsies of the right and left hepatic lobes revealed a statistically significant discordance in steatosis measurements (>20% of hepatocytes affected), inflammation (>1 grade), and fibrosis (>1 stage) in 18%, 41%, and 43% of the patients, respectively (10). Multiple biopsy passes seem to lead to a more accurate diagnosis (64).

Liver biopsy may not be proposed as a screening procedure because it is an invasive technique associated with life-threatening complications in both adults and children; however, it has recently been shown that this procedure can be carried out safely in obese children with no increase in complication rate compared with nonobese children (62,65,66). Its high cost is also a problem.

Another consideration is the timing for liver biopsy. Some investigators recommend performing liver biopsy before starting any type of pharmacological treatment (62). A practical approach could be to wait 6 months while awaiting the results of dietary and/or exercise as intervention. If no response on biochemical and/or ultrasonographic NAFLD surrogate markers is obtained, then one should consider histological evaluation (26,67). The European Association for the Study of Liver suggests performing a biopsy in adult patients in whom advanced fibrosis is suspected and avoiding biopsies in patients who are actively undergoing lifestyle modifications (68).

It seems reasonable that in pediatrics liver biopsy should be indicated to exclude treatable disease, in cases of clinically suspected advanced liver disease, before pharmacological/surgical treatment, and as part of a structured intervention protocol or clinical research trial. No evidence-based recommendation, however, exists in this regard. Roberts et al (19) proposed biopsy criteria for children affected by NAFLD. These included young age (<10 years), family history of severe NAFLD, presence of hepatosplenomegaly at physical examination, and abnormal laboratory results. The latter include marked and persistent hypertransaminasemia, severe insulin resistance, presence of nonorgan-specific autoantibodies, and inconclusive results from biochemical tests for other liver pathologies such as Wilson disease. Children with hypothalamic dysfunction have been shown to be at risk for a rapid rate of NAFLD progression, and therefore may justify a liver biopsy. There is no present consensus or evidence base to advise on the timing of or necessity for subsequent histological monitoring.

Executive Summary

Liver biopsy is required for definitive diagnosis of NAFLD. Due to its invasive nature and high cost, liver biopsy is not proposed as a screening procedure. Indications for liver biopsy are still discussed and there is no present consensus or evidence base to formulate guidelines. Indications for liver biopsy are based on expert opinions and take into consideration differential diagnosis and the risk of progression of liver disease to cirrhosis.

The panel accepted in general the criteria of Roberts et al and summarized indications for liver biopsy as follows: to exclude other treatable disease, in cases of clinically suspected advanced liver disease, before pharmacological/surgical treatment, and as part of a structured intervention protocol or clinical research trial. For differential diagnosis, liver biopsy should be considered the last test after noninvasive biochemical and metabolic tests.

IMAGING METHODS

Table 4 shows a comparison of the main available imaging techniques versus liver biopsy. Most of these studies refer to adult NAFLD because there are no studies performed on large samples of children with NAFLD (69,70).

Ultrasonography is the most common imaging technique used for NAFLD screening because it is safe, widely available, relatively inexpensive, and can detect any evidence of portal hypertension (69). Steatosis appears as a bright or hyperechoic liver as compared with the adjacent right kidney or spleen. The degree of fatty infiltration is visually assessed by degree of echogenicity. Echogenicity is measured as a progressive increase in fine echoes of liver parenchyma (grade 0–3 compared with intrahepatic vessel borders). Posterior attenuation and/or skip areas are closely related to steatosis >30%. As shown in Table 4, when compared with liver biopsy in the adult population, this technique has a sensitivity ranging from 60% to 96% and a specificity ranging from 84% to 100% (71,72). When the percentage of steatosis is ≥20%, sensitivity and specificity increase to 100% and 90%, respectively (72); however, early studies reported a lower sensitivity when fat content was <30% (73). In children its accuracy has been evaluated only in 1 study that reported comparable results (74). Other limitations of US are that it is operator dependent and that it does not easily distinguish liver steatosis from fibrosis. More recently, Palmentieri et al (75) reported that in their hands a bright liver echo pattern is associated only with steatosis and not with fibrosis. US hepatic-renal ratio has been shown to accurately measure fat content compared with magnetic resonance spectroscopy (MRS) (76) and has not been fully validated in children (77). Finally, it is evident that when fatty liver is suspected by US, a wide series of causes of liver steatosis other than NAFLD need to be excluded on clinical and laboratory bases (Table 2).

Unenhanced computed tomography (CT) is a more specific technique than US for the quantitative detection of fatty liver. The CT diagnosis of hepatic steatosis is made by measuring the difference in liver and spleen attenuation values expressed in Hounsfields units. In a study in adults with NAFLD, CT sensitivity and specificity compared with liver biopsy were 82% and 100%, respectively (78) (Table 4). The authors concluded that the diagnostic performance of unenhanced CT in the quantitative assessment of macrovesicular steatosis is not clinically accepted. This feature, together with unnecessary exposure to ionizing radiations, limit the potential use of CT in longitudinal studies, especially in children.

MRI, using the double-gradient echo chemical shift imaging technique, increasingly has been shown to reliably measure fat infiltration of the liver. This method differentiates tissues

containing only water from those containing both fat and water. This noninvasive and nonirradiating technique is of great interest for use in children. At the moment, limited data are available regarding the utility of hepatic fat quantification in pediatric NAFLD (79). A study involving 50 obese children with NAFLD showed good correlation between MRI and US ($P < 0.0001$), especially in patients with moderate and severe steatosis (80). In that study, however, there was no comparison with liver biopsy. In adults, sensitivity and specificity of MRI compared with histopathological findings are 100% and 90.4%, respectively (81) (Table 4). Furthermore, MRI is not subject to interobserver variation and may be more useful than US for monitoring children with fatty liver. It can prove useful in identifying fat regression or progression, especially when the grade is mild (82). Novel approaches have been proposed to improve the accuracy of the technique (83).

An emerging imaging modality for the quantitative assessment of hepatic steatosis is **1H-MR spectroscopy (1H-MRS)**. This technique grades hepatic triglyceride content by directly measuring protons in the acyl groups of the liver tissue triglycerides. Sensitivity and diagnostic precision in adults range from 87% to 100% and from 80% to 85%, respectively (84). MRS has been applied successfully in a pediatric pilot study to measure hepatic fat content in patients with biopsy-proven NASH before and after pharmacological treatment (85). At present, it is the most accurate method in which fat content is <10%, and a recent meta-analysis confirmed that MRI and 1H-MRS are the techniques of choice for accurate evaluation of steatosis (86); however, MRS is not widely performed because it is time-consuming and requires off-scan analysis by an expert. Because of these limitations, MRS at present seems to be most appropriate for research studies at specialized centers and is not suitable for widespread use.

The **fibroscan** is a new medical device using transient elastography to evaluate liver fibrosis based on stiffness in a noninvasive, rapid, painless, and reproducible way. Promising results were first shown in adults with chronic hepatitis C (87). Fibroscan has correlated well with hepatic histology both in adults (88) and in children (70) with chronic liver disease including NAFLD. In adults, sensitivity ranges from 81% to 85%, and specificity from 74% to 78%. (89) (Table 4). The limitation of this technique is that fibrosis may be mistaken for steatosis in adult patients with a BMI >28 (89). In addition, this technique is not yet performed in everyday clinical practice. Finally, its resolution is not sufficient to detect changes in fibrosis over time and after treatment.

A few studies have demonstrated the accuracy of this technique in assessing hepatic fibrosis in children with NAFLD. Unfortunately, at the present time, the probe size is not appropriate for smaller children (70,88). Most recently normal values of liver stiffness in children ages 7.5 to 8.6 years, using a pediatric probe, have been proposed (90). The results showed that a wide difference exists between values obtained using adult and pediatric probes. Further validation of transient elastography is necessary before it can be adopted for the stratification of NAFLD in childhood either alone or in association with other noninvasive approaches.

Magnetic resonance elastography (MRE) uses a modified phase-contrast MRI sequence to visualize propagating share waves in tissues. It could be a convenient complement to MRS to estimate noninvasively the degree of steatosis and degree of liver stiffness; however, further studies are required before MRE can be introduced in clinical practice. Its association with laboratory tests (eg, AST/platelet ratio index, GGT levels) may improve the specificity and sensitivity of the noninvasive estimation of liver fibrosis (46,91). At the moment there are no data concerning the use of MRE in children. Therefore, we consider this technique to be experimental.

Executive Summary

Accurate noninvasive imaging techniques to diagnose and monitor NAFLD are being developed, as follows:

1. *Ultrasounds are safe, but they are limited by the inability to quantify steatosis or fibrosis.*
2. *MRI is not cost-effective, even if with certain modifications it could enable rapid, reproducible measurements of steatosis and fibrosis.*
3. *Fibroscan has been used in children with NAFLD, but in its present state of development it is not yet suitable for widespread use in these patients.*

NOVEL NONINVASIVE LABORATORY ASSESSMENT OF NAFLD STAGES AND GRADES

Despite the high prevalence of pediatric NAFLD, few studies have evaluated noninvasive markers for the prediction of hepatic steatosis and progression to steatohepatitis in children. Logical markers would reflect the most common pathogenic mechanisms underlying NAFLD. Some of these are highlighted here.

Serum Markers of Hepatic Inflammation

An imbalance between several proinflammatory (TNF-α; resistin, interleukin-6) and anti-inflammatory cytokines (adipokines [eg, adiponectin]) seems to be involved in the progression from simple steatosis to NASH (92). Therefore, a variety of biomarkers of hepatic inflammation have been proposed as surrogate markers for diagnosing NASH both in adult and pediatric ages.

Low serum levels of adiponectin have been reported in pediatric patients with NASH with normal levels of proinflammatory cytokines, suggesting that adiponectin may play a role in both the pathogenesis and disease progression (93). As such, it may serve as a noninvasive biomarker.

Serum leptin has been implicated in disease progression because of a direct profibrotic effect (94). High levels of serum TNF-α (a major inflammatory cytokine that is also secreted by adipose tissue, and antagonizes the effects of adiponectin) and serum leptin correlated well with histological features of steatohepatitis in a cohort of histologically proven NAFLD in children (95).

Retinol-binding protein 4, another adipokine that is associated with insulin resistance, has been shown in a pediatric cohort of 59 children with biopsy-proven NAFLD to be inversely correlated to degree of liver damage (96).

A recent study in a pediatric cohort with biopsy-proven NAFLD showed that serum levels of endotoxin and plasminogen activator inhibitor-1 may serve as a reliable marker of NASH, suggesting that endotoxin may participate in the progression from NAFLD to NASH (97).

Although several groups have investigated circulating cytokine levels and their correlation with disease severity, there are no data at the present time supporting their generalized use in the diagnosis of NASH in adults and children.

Another inflammatory biomarker that seems involved in NAFLD progression is C-reactive protein, an acute-phase reactant synthesized in the liver. This marker is frequently elevated in subjects with metabolic syndrome and represents an independent predictor of NAFLD (32). High-sensitivity C-reactive protein has been reported as a potential marker of severity of fibrosis in adult NASH (98).

Increased plasma ferritin levels are seen in 20% to 50% of adults with NAFLD and elevated transferrin saturation in 5% to 10% (99). These data also have been reported in pediatric patients with NAFLD as a marker of systemic inflammation rather than a marker of iron overload (32). An association with a heterozygosity for human hemochromatosis (*HFE*) gene has been described both in adults (100) and in children (32).

Another important mediator of hepatic insulin resistance, fetuin-A, has been described in children with metabolic syndrome and fatty liver. Fetuin-A was significantly higher in obese children with NAFLD versus controls and decreased considerably in those who lost weight, suggesting its role as a potential biomarker for diagnosis and assessment of treatment response (101).

Markers of Oxidative Stress

Enhanced oxidative stress (OS) has long been recognized as a mechanism involved in liver damage and disease progression in human and animal models of NASH. Several oxidation pathways may play a role in the overproduction of reactive oxygen species. A number of studies have attempted to elucidate whether measurement of systemic markers of OS may reflect the levels of OS present in the liver (102).

Chalasani et al (103) measured circulating levels of lipid peroxidation products and their metabolic and nutritional correlates in 21 adults with NASH and 19 matched controls. Although patients with NASH had higher serum levels of oxidized low-density lipoprotein and thiobarbituric acid–reacting substance, these differences were not significant in multivariate analyses and total antioxidant status did not differ between groups. In 59 children with NAFLD versus controls and hepatitis C virus, hepatic lipid peroxidation (studied as hepatic malondialdehyde) was increased, but without differences in NAFLD versus NASH (104). In another study involving 36 children with NAFLD, increased serum levels of protein glutathionylation were detected, showing a correlation of this OS marker with histological steatohepatitis and liver fibrosis (105). Similar results were seen in 40 children with biopsy-proven NAFLD in which OS was evaluated with serum protein carbonyls, hepatic expression of 8-hydroxy-2-deoxyguanosine, and circulating antibody against malondialdehyde-adducted human serum albumin (106).

Bilirubin has known antioxidant properties, and variants in the *UGT1A1* gene (Gilbert syndrome) contribute to increased bilirubin levels and reduced risk for onset or development of NAFLD (16).

Markers of Apoptosis

Several groups have reported that serum markers of hepatocyte apoptosis can discriminate NASH from benign steatosis. Cytokeratin 18 (CK18) is an intracellular intermediate filament protein expressed at high levels by hepatocytes. Caspase-cleaved CK18 fragments represent an indirect measure of cell death; high serum levels have been reported in adults with NASH and these levels correlate well with disease stage on biopsy (107). In a pediatric study, Vos et al (108) showed that CK18 levels are elevated in patients with NAFLD compared with normal-weight controls and obese individuals without liver involvement. Levels in NASH were higher than in simple steatosis without reaching statistical significance. A more recent work showed instead that CK18 M30 levels were significantly higher in patients with NAFLD than in controls (median 288 vs 172 IU/L) and in those with steatohepatitis (median 347 IU/L) versus simple steatosis (NAS <3; median 191 IU/L). Significant fibrosis (≥F2) could be differentiated from no/minimal fibrosis (<F2) (median 393 vs 243 IU/L) (109). Further studies in the pediatric population are necessary to confirm the usefulness of this marker in stratifying disease severity with its potential use in the longitudinal monitoring of disease progression in pediatric NAFLD.

Markers of Hepatic Fibrosis

The increasing number of children with NAFLD is anticipated to lead sooner or later to the increased prevalence of liver cirrhosis and hepatocellular carcinoma. Several noninvasive approaches have been proposed as a replacement for, or to be used with, the histopathological analysis of liver biopsy.

A combination of several demographic, anthropometric, and laboratory features has been assessed both in adults and children as predictors of liver fibrosis. Among demographic factors, age was significantly associated with the presence of fibrosis in a pediatric study (62), but it was not in a previous study (21). In these studies, children with (perisinusoidal) fibrosis tended to be more obese than children without fibrosis. Children with moderate fibrosis may have a greater degree of insulin resistance than those with mild fibrosis (22,26).

Nonspecific Markers of Liver Fibrosis

An AST/ALT ratio >1 may indicate advanced fibrosis, but sensitivity is poor (26). High AST/platelet ratio index is promising in adults (110) but requires further validation in children with NAFLD. As previously mentioned, high levels of GGT are associated with histological findings of advanced fibrosis (22,26). Recently, waist circumference is the only component of the metabolic syndrome to have been shown to contribute to liver fibrosis in children with NASH (38).

An increasing number of serum fibrosis markers panels have been introduced in the last several years, reflecting the lack of validated noninvasive measures of hepatic fibrosis in NAFLD (111). Most of the studies were conducted in adults. The most widely validated fibrosis marker panel is the FibroTest (including total bilirubin, GGT, α2 macroglobulin, apolipoprotein A1, haptoglobin, corrected for age and sex; Biopredictive, Paris, France) (112). This panel, originally described in patients with chronic hepatitis C virus, has shown good correlation with liver biopsy results in patients with NAFLD (89). Another panel, the FIB4 index (based on age, AST and ALT levels, and platelet counts), has been shown to be superior to 7 other noninvasive markers of fibrosis in adult patients with NAFLD (113). A noninvasive index called the pediatric NAFLD fibrosis index calculated based on age, waist circumference, and triglycerides seems to predict the presence of fibrosis in children with NAFLD, but still needs to be cross-validated, especially for longitudinal assessment of fibrotic changes (114,115) (Table 5).

Fibrosis Markers

The European liver fibrosis (ELF) panel, which includes hyaluronic acid (HA), amino-terminal propeptide of type III collagen, and tissue inhibitor of metalloproteinase I, has been proposed as a screening test for progressive fibrosis, with a good predictive capability. A study assessed ELF score in predicting liver fibrosis in children with NAFLD. It showed a high degree of sensitivity and specificity, compared with liver biopsy (115) (Table 5). The combination of ELF panel and NAFLD fibrosis index in children was significantly better in differentiating any fibrosis from no fibrosis with an AUC of 0.944 (95% CI 0.917–0.99) (116).

TABLE 5. Fibrosis markers panels evaluated in children with NAFLD

Test (reference)	Age	Components	Proprietary	AUROC for F2–4 vs F0–1	Sensitivity	Specificity	Comparison
PNFI in pediatric age (114)	P	Age, waist circumference, triglycerides	No	0.85 (0.80–0.90)	PPV 98.5% (PNFI >9)	NPV 44.5% (PNFI >9)	Histology
ELF in pediatric age (115)	P	Hyaluronic acid, TIMP-1, PIII-NP	No	0.98 (0.96–1.00)	94%	93%	Histology

AUROC = area under receiver operator curve; ELF = European liver fibrosis; P = pediatric patients; PNFI = pediatric NAFLD fibrosis Index; PIII-NP = N-terminal peptide of procollagen III; TIMP I = tissue inhibitor of metalloproteinase I.

In children, serum HA and/or laminin has already been shown to be promising for the prediction of hepatic fibrosis in children with other chronic hepatopathies (117). It has been shown that the serum levels of HA alone are predictors of the degree of hepatic fibrosis in children with NAFLD (118). If these data are confirmed by further studies, HA may allow a simple and efficient screening of patients at risk for progressive liver disease needing further investigation, including the execution of liver biopsy, in specialized centers. The combination of HA and tissue inhibitor of metalloproteinase I with clinical variables (age) seemed to represent a reliable noninvasive serum marker of fibrosis + inflammation that predicted the presence of NASH in a pilot cohort of adults with NAFLD (119).

OTHER BIOCHEMICAL PREDICTORS

Poynard et al (120) reported a combination of markers for steatosis referred to as the Steatotest. This test combines 10 blood components readily available in every laboratory (cholesterol, triglycerides, glucose, AST, ALT, GGT, bilirubin, haptoglobin, $\alpha2$ macroglobulin, and apolipoprotein A1) with age, sex, and BMI. Concordance of this test with biopsy for steatosis detection has been demonstrated to be higher than US ($P = 0.02$) and more sensitive for the follow-up of treated patients. Recently, the same group also developed the NashTest, which includes AST in addition to the components of the SteatoTest. This panel has demonstrated good sensitivity and high specificity in a large cohort of adult patients for the diagnosis of NASH versus no NASH or borderline cases as compared with the NAS (121). A study with 2 concomitant percutaneous liver biopsies in patients with NAFLD showed a high risk of misdiagnosis of NASH and fibrosis staging with a lack of accuracy versus liver biopsy, probably also because the histological lesions could be unevenly distributed throughout the liver parenchyma (10). Finally, the FibroMax Test (FibroTest + Steato-SteatoTest + NashTest) (122) has been introduced in adults as a simple, noninvasive marker that indirectly estimates histological findings. At the moment it needs further investigation to establish its accuracy, especially in the pediatric age group. These predictive tests (generally reaching an AUC of approximately 0.80 ± 0.10 in adults) could limit the need for liver biopsy (115) because of its inherent risk of sampling error.

Executive Summary

Development of noninvasive methods is needed to identify children with NAFLD and predict those at increased risk for progression to NASH.

Markers of inflammation, OS, apoptosis, and fibrosis have been reported from several groups trying to discriminate NASH from benign steatosis. The panel agreed that larger groups are needed to validate these reported diagnostic test characteristics in
pediatric fatty liver disease before they can be applied in clinical practice.

FUTURE RESEARCH DIRECTIONS

Any future research direction should take account of proteomic methodologies that include the isolation, identification, and quantification of proteins by means of surface-enhanced laser desorption/ionization time-of-flight mass spectrometry (123). The identification of novel proteins (eg, related to double-charged ions of α- and β-hemoglobin subunits) by proteomic profiling predictive of NASH in obesity will probably be helpful for the definition of NAFLD disease subphenotypes and for the response to therapy. In a study involving 69 patients with varying stages of NAFLD among >1700 identified serum proteins, expression levels of 55 and 15 proteins changed significantly between the simple steatosis and NASH F3/F4 group and the overall NASH plus NASH F3/F4 group, respectively. Classification of proteins with significant changes showed an involvement in immune system regulation and inflammation, coagulation, cellular and extracellular matrix structure and function, and roles as carrier proteins in the blood. Furthermore, many of these proteins are synthesized exclusively by the liver and could serve as diagnostic biomarkers for identifying and staging NAFLD (124).

It is hoped that growing information on the underlying susceptibility of genetic factors to NASH and/or type of prognosis in affected individuals will lead to a reduction in the rate of liver biopsies to screen for NASH and will probably prove useful in the management of at-risk obese children for early diagnosis, monitoring, and tailored treatments.

Executive Summary

NASH is a complex multifactorial metabolic disease as a consequence of a wide variety of insults. Improved understanding of pathogenesis through genetic and familial studies is a challenge for the near future. Strategic information will come from large population-based epidemiological studies ongoing around the world. Newly developed techniques in the proteomic field may help us to identify validated criteria in the selection of children who should undergo more extensive evaluation.

CONSIDERATIONS AND CONCLUSIONS

NAFLD is still underdiagnosed in children. Its recognition is based on detection of fatty liver combined with risk factors (mainly central obesity/overweight) and exclusion of other liver diseases. The reference but imperfect standard for confirming liver steatosis and/or NASH is liver biopsy, which has important limitations, including its risks/complications, cost, and possible sampling error. Some noninvasive surrogate markers have been developed aimed at assessing the degree of steatosis, inflammation and fibrosis, and the

risk of progression to end-stage liver disease. Among these, liver imaging (ultrasonography or MRI), liver function tests (ie, transaminases ratio, GGT levels), and specific or nonspecific markers of liver fibrosis are used increasingly. Their diagnostic accuracy compared with liver biopsy has been evaluated in children rarely and further studies are needed to establish the sensitivity and specificity of these methods in this age range. In the future, the increasing understanding of the pathogenic mechanisms underlying disease progression of NAFLD will, it is hoped, result in the development of new, specific markers. Such a development could represent a valid alternative to liver biopsy at initial assessment and during follow-up, and should facilitate individualized medical treatment.

For clinical purposes, the diagnosis of NAFLD is at present usually based on presence of ≥1 features of the metabolic syndrome (mostly in children older than 10 years), ultrasound imaging of the liver showing liver brightness, and eventually increased transaminase activity. Exclusions of other steatotic or nonsteatotic liver diseases are mandatory in pediatrics, and the performed tests and procedures should be adjusted to age and clinical presentation. Therefore, it is difficult to create a simple diagnostic algorithm as it was proposed for adults (69,125). Still, some major indications should be provided to pediatricians taking care of obese/overweight children. A stepwise diagnostic approach based on the hitherto examined studies is proposed below.

Diagnostic Algorithm

NAFLD should be suspected in overweight/obese children and adolescents, especially if there is familial clustering, if they consume drinks with high fructose content, and if their waist circumference is >>95th percentile for age and sex. Abdominal US and liver function tests should be performed in this group of children. Finding increased transaminase activity and/or bright liver on US mandate further investigations to exclude other major causes of liver disease, including infectious hepatitis, autoimmune hepatitis, liver toxic injury, Wilson disease, and α_1-antitrypsin deficiency. Increased serum ALT and GGT raise the suspicion of NAFLD in children at risk for more severe disease. Overweight/ obese children with normal US imaging and normal liver function tests still should be studied in obesity clinics, and any abnormal findings on physical examination indicating liver disease, appearance of other complications of obesity, and increasing obesity should be considered for further investigation for NAFLD. Although NAFLD is less common at younger ages, US imaging and liver function tests should also be performed in obese children ages 3 to 10 years. Because severe liver injury due to NAFLD is rare at this age, abnormal imaging or liver function tests warrant thorough workup and exclusion of age-specific diagnoses.

Overweight/obesity below 3 years of age usually does not produce liver steatosis. Consequently, it is our opinion that children in this age group do not need to be screened for NAFLD. Still, brightness of the liver or increased aminotransferases in this age group requires a detailed workup including the many rare metabolic or systemic diseases presenting with fatty liver (the so-called NASH trash bin) (20,126,127). The proposed diagnostic approach to children with overweight/obesity and/or abnormal liver function tests is presented in a flowchart (Fig. 1).

The major consideration of a clinician is the right time and indications for liver biopsy, which were also described in the flowchart. We recommend following the indications of Roberts et al (19) for liver biopsy in children possibly affected by NAFLD. These included young age (younger than 10 years), family history of severe NAFLD, presence of hepatosplenomegaly at physical

examination, and abnormal laboratory results. Among these are persistent hypertransaminasemia, insulin resistance (measured by HOMA-IR), nonorgan-specific autoantibodies, and inconclusive results from biochemical tests for severe/progressive liver diseases such as Wilson disease. Among the other criteria warranting liver biopsy, one may consider the association of other liver diseases such as chronic viral hepatitis and α_1-antitrypsin deficiency. Furthermore, hypothalamic expansive processes have been shown to be associated with a rapid rate of NAFLD progression and therefore may justify a liver biopsy. Finally, it is accepted that biopsies should be performed before initiating pharmacological treatment.

Fibrosis markers evaluated at the same time as liver biopsy may be useful in the near future to follow-up fibrotic changes without the need for repeating histology.

REFERENCES

1. Loomba R, Sirlin CB, Schwimmer JB, et al. Advances in pediatric nonalcoholic fatty liver disease. *Hepatology* 2009;50:1282–93.
2. Schwimmer JB, Deutsch R, Kahen T, et al. Prevalence of fatty liver in children and adolescents. *Pediatrics* 2006;118:1388–93.
3. Feldstein AE, Charatcharoenwitthaya P, Treeprasertsuk S, et al. The natural history of nonalcoholic fatty liver disease in children: a follow-up study for up to 20 years. *Gut* 2009;58:1538–44.
4. Molleston JP, White F, Teckman J, et al. Obese children with steatohepatitis can develop cirrhosis in childhood. *Am J Gastroenterol* 2002;97:2460–2.
5. Socha P, Horvath A, Vajro P, et al. Pharmacological interventions for nonalcoholic fatty liver disease in adults and in children: a systematic review. *J Pediatr Gastroenterol Nutr* 2009;48:587–96.
6. Kistler KD, Molleston J, Unlap A, et al. Symptoms and quality of life in obese children and adolescents with nonalcoholic fatty liver disease. *Aliment Pharmacol Ther* 2010;31:396–406.
7. Socha P, Wierzbicka A, Dhawan A. Non alcoholic fatty liver disease in children. In: Jara P, ed. *International Hepatology Updates: Liver Diseases in Children.* Barcelona, Spain: Perlmayer; 2009:147–161.
8. De Bruyne RM, Fitzpatrick E, Dhawan A. Fatty liver disease in children: eat now pay later. *Hepatol Int* 2010;4:375–85.
9. Wieckowska A, McCullough AJ, Feldstein AE. Noninvasive diagnosis and monitoring of nonalcoholic steatohepatitis: present and future. *Hepatology* 2007;46:582–9.
10. Ratziu V, Charlotte F, Heurtier A, et al. Sampling variability of liver biopsy in nonalcoholic fatty liver disease. *Gastroenterology* 2005; 7:1898–906.
11. Younossi ZM, Baranova A, Ziegler K, et al. A genomic and proteomic study of the spectrum of nonalcoholic fatty liver disease. *Hepatology* 2005;42:665–74.
12. Santoro N, Kursawe R, D'Adamo E, et al. A common variant in the patatin-like phospholipase 3 gene (PNPLA3) is associated with fatty liver disease in obese children and adolescents. *Hepatology* 2010; 4:1281–90.
13. Romeo S, Sentinelli F, Cambuli VM, et al. The 148 M allele of the PNPLA3 gene is associated with indices of liver damage early in life. *J Hepatol* 2010;53:335–8.
14. Valenti L, Alisi A, Galmozzi E, et al. I148 M patatin-like phospholipase domain-containing 3 gene variant and severity of pediatric nonalcoholic fatty liver disease. *Hepatology* 2010;4:1274–80.
15. Carulli L, Canedi I, Rondinella S, et al. Genetic polymorphisms in nonalcoholic fatty liver disease: interleukin-6-174G/C polymorphism is associated with non-alcoholic steatohepatitis. *Dig Liver Dis* 2009; 41:823–8.
16. Tokushige K, Takakura M, Tsuchiya-Matsushita N, et al. Influence of TNF gene polymorphisms in Japanese patients with NASH and simple steatosis. *J Hepatol* 2007;46:1104–10.
17. Miele L, Beale G, Patman G, et al. The Kruppel-like factor 6 genotype is associated with fibrosis in nonalcoholic fatty liver disease. *Gastroenterology* 2008;135:282–91.
18. Lin YC, Chang PF, Hu FC, et al. Variants in the *UGT1A1* gene and the risk of pediatric nonalcoholic fatty liver disease. *Pediatrics* 2009; 124:e1221–7.

19. Roberts EA. Pediatric nonalcoholic fatty liver disease (NAFLD): a "growing" problem? *J Hepatol* 2007;46:1133–42.

20. Cassiman D, Jaeken J. NASH may be trash. *Gut* 2008;57:141–4.

21. Schwimmer JB, Deutsch R, Rauch JB, et al. Obesity, insulin resistance, and other clinicopathological correlates of pediatric nonalcoholic fatty liver disease. *J Pediatr* 2003;143:500–5.

22. Schwimmer JB, Behling C, Newbury R, et al. Histopathology of pediatric nonalcoholic fatty liver disease. *Hepatology* 2005;42:641–9.

23. Louthan MV, Theriot JA, Zimmerman E, et al. Decreased prevalence of nonalcoholic fatty liver disease in black obese children. *J Pediatr Gastroenterol Nutr* 2005;4:426–9.

24. Willner IR, Waters B, Patil SR, et al. Ninety patients with non alcoholic steatohepatitis: insulin resistance, familial tendency, and severity of disease. *Am J Gastroenterol* 2001;96:2957–61.

25. Schwimmer JB, Celedon MA, Lavine JE, et al. Heritability of non alcoholic fatty liver disease. *Gastroenterology* 2009;136:1585–92.

26. Patton HM, Lavine JE, Van Natta ML, et al. Clinical correlates of histopathology in pediatric nonalcoholic steatohepatitis. *Gastroenterology* 2008;135:1961–71.

27. Franzese A, Vajro P, Argenziano A, et al. Liver involvement in obese children. Ultrasonography and liver enzyme levels at diagnosis and during follow-up in an Italian population. *Dig Dis Sci* 1997; 42:1428–32.

28. Nobili V, Alisi A, Panera N, et al. Low birth weight and catch-up-growth associated with metabolic syndrome: a ten year systematic review. *Pediatr Endocrinol Rev* 2008;6:241–7.

29. Nobili V, Bedogni G, Alisi A, et al. A protective effect of breastfeeding on the progression of NAFLD. *Arch Dis Child* 2009;94:801–5.

30. Abdelmalek MF, Suzuki A, Guy C, et al. Increased fructose consumption is associated with fibrosis severity in patients with nonalcoholic fatty liver disease. *Hepatology* 2010;51:1961–71.

31. Ahmed MH, Byrne CD. Obstructive sleep apnea syndrome and fatty liver: association or causal link? *World J Gastroenterol* 2010; 16:4243–52.

32. Mandato C, Lucariello S, Licenziati MR, et al. Metabolic, hormonal, oxidative, and inflammatory factors in pediatric obesity-related liver disease. *J Pediatr* 2005;147:62–6.

33. Taylor RW, Jones IE, Williams SM, et al. Evaluation of waist circumference, waist-to-hip ratio, and the conicity index as screening tools for high trunk fat mass, as measured by dual-energy X-ray absorptiometry, in children aged 3–19 y. *Am J Clin Nutr* 2000; 72:490–5.

34. Moreno LA, Pineda I, Rodríguez G, et al. Waist circumference for the screening of the metabolic syndrome in children. *Acta Paediatr* 2002;91:1307–12.

35. McCarthy HD, Jarrett KV, Crawley HF. The development of waist circumference percentiles in British children aged 5.0–16.9 years. *Eur J Clin Nutr* 2001;55:902–7.

36. Katzmarzyk PT. Waist circumference percentiles for Canadian youth 11–18 y of age. *Eur J Clin Nutr* 2004;58:1011–5.

37. Lin YC, Chang PF, Yeh SJ, et al. Risk factors for liver steatosis in obese children and adolescents. *Pediatr Neonatol* 2010;51:149–54.

38. Manco M, Bedogni G, Marcellini M, et al. Waist circumference correlates with liver fibrosis in children with non-alcoholic steatohepatitis. *Gut* 2008;9:1283–7.

39. Vajro P, Fontanella A, Perna C, et al. Persistent hyperaminotransferasemia resolving after weight reduction in obese children. *J Pediatr* 1994;125:239–41.

40. Nobili V, Reale A, Alisi A, et al. Elevated serum ALT in children presenting to the emergency unit: relationship with NAFLD. *Dig Liver Dis* 2009;41:749–52.

41. Manco M, Alisi A, Nobili V. Risk of severe liver disease in NAFLD with normal ALT levels: a pediatric report. *Hepatology* 2008; 48:2087–8.

42. Burgert TS, Taksali SE, Dziura J, et al. Alanine aminotransferase levels and fatty liver in childhood obesity: associations with insulin resistance, adiponectin, and visceral fat. *J Clin Endocrinol Metab* 2006;91:4287–94.

43. Schwimmer JB, Dunn W, Norman GJ, et al. SAFETY study: alanine aminotransferase cutoff values are set too high for reliable detection of pediatric chronic liver disease. *Gastroenterology* 2010;138:1357–64.

44. England K, Thorne C, Pembrey L, et al. Age- and sex-related reference ranges of alanine aminotransferase levels in children: European Paediatric HCV Network. *J Pediatr Gastroenterol Nutr* 2009;49:71–7.

45. Fishbein MH, Miner M, Mogren C, et al. The spectrum of fatty liver in obese children and the relationship of serum aminotransferases to severity of steatosis. *J Pediatr Gastroenterol Nutr* 2003;36:54–61.

46. Tahan V, Canbakan B, Balci H, et al. Serum gamma-glutamyl-transpeptidase distinguishes non-alcoholic fatty liver disease at high risk. *Hepatogastroenterology* 2008;55:1433–8.

47. Sundaram SS, Zeitler P, Nadeau K. The metabolic syndrome and nonalcoholic fatty liver disease in children. *Curr Opin Pediatr* 2009; 21:529–35.

48. Cambuli VM, Incani M, Pilia S, et al. Oral glucose tolerance test in Italian overweight/obese children and adolescents results in a very high prevalence of impaired fasting glycaemia, but not of diabetes. *Diabetes Metab Res Rev* 2009;25:528–34.

49. Sartorio A, Del Col A, Agosti F, et al. Predictors of non-alcoholic fatty liver disease in obese children. *Eur J Clin Nutr* 2007;61:877–83.

50. Oliveira AM, Oliveira N, Reis JC, et al. Triglycerides and alanine aminotransferase as screening markers for suspected fatty liver disease in obese children and adolescents. *Horm Res* 2009;71:83–8.

51. Engiz O, Berberoğlu M, Siklar Z, et al. Risk factors for non-alcoholic fatty liver disease in obese children. *Horm Res* 2009;72:63–4.

52. Nobili V, Alkhouri N, Bartuli A, et al. Severity of liver injury and atherogenic lipid profile in children with nonalcoholic fatty liver disease. *Pediatr Res* 2010;67:665–70.

53. Lonardo A, Loria P, Leonardi F, et al. Fasting insulin and uric acid levels but not indices of iron metabolism are independent predictors of non-alcoholic fatty liver disease. A case-control study. *Dig Liver Dis* 2002;34:204–11.

54. Germenis AE, Yiannaki EE, Zachou K, et al. Prevalence and clinical significance of immunoglobulin A antibodies against tissue transglutaminase in patients with diverse chronic liver diseases. *Clin Diagn Lab Immunol* 2005;12:941–8.

55. Franzese A, Iannucci MP, Valerio G, et al. Atypical celiac disease presenting as obesity-related liver dysfunction. *J Pediatr Gastroenterol Nutr* 2001;33:329–32.

56. Adams LA, Lindor KD, Angulo P. The prevalence of autoantibodies and autoimmune hepatitis in patients with nonalcoholic fatty liver disease. *Am J Gastroenterol* 2004;99:1316–20.

57. Veropalumbo C, Del Giudice E, Capuano G, et al. Duchenne and Becker muscular dystrophy presenting as nonalcoholic fatty liver disease. *J Pediatr Gastroenterol Nutr* 2011;53:463–4.

58. Spindelboeck W, Deutschmann A, Lackner K, et al. Obesity and Wilson disease in childhood and adolescence. *J Pediatr Gastroenterol Nutr* 2009;48(suppl 3):E63.

59. Kleiner DE, Brunt EM, Van Natta M, et al. Design and validation of a histological scoring system for nonalcoholic fatty liver disease. *Hepatology* 2005;41:1313–21.

60. Brunt EM. Pathology of nonalcoholic fatty liver disease. *Nat Rev Gastroenterol Hepatol* 2010;7:195–203.

61. Brunt EM, Kleiner DE, Wilson LA, et al. Portal chronic inflammation in nonalcoholic fatty liver disease (NAFLD): a histologic marker of advanced NAFLD-clinicopathologic correlations from the nonalcoholic steatohepatitis clinical research network. *Hepatology* 2009;49:809–20.

62. Nobili V, Marcellini M, Devito R, et al. NAFLD in children: a prospective clinical-pathological study and effect of lifestyle advice. *Hepatology* 2006;44:458–65.

63. Carter-Kent C, Yerian LM, Brunt EM, et al. Nonalcoholic steatohepatitis in children: a multicenter clinicopathological study. *Hepatology* 2009;48:1113–20.

64. Vuppalanchi R, Unalp A, Van Natta M, et al. Effects of liver biopsy sample length and number of readings on sampling variability in nonalcoholic fatty liver disease. *Clin Gastroenterol Hepatol* 2009; 4:481–6.

65. Pietrobattista A, Fruwirth R, Natali G, et al. Is juvenile liver biopsy unsafe? Putting an end to a common misapprehension. *Pediatr Radiol* 2009;39:959–61.

66. Harwood J, Bishop P, Liu H, et al. Safety of blind percutaneous liver biopsy in obese children: a retrospective analysis. *J Clin Gastroenterol* 2010;44:e253–5.

67. Manco M, Bottazzo G, DeVito R, et al. Nonalcoholic fatty liver disease in children. *J Am Coll Nutr* 2008;27:667–76.

68. Ratziu V, Bellentani S, Cortez-Pinto H, et al. A position statement on NAFLD/NASH based on the EASL 2009 special conference. *J Hepatol* 2010;53:372–84.

69. Roldan-Valadez E, Favila R, Martínez-López M, et al. Imaging techniques for assessing hepatic fat content in nonalcoholic fatty liver disease. *Ann Hepatol* 2008;7:212–20.

70. Nobili V, Vizzutti F, Arena U, et al. Accuracy and reproducibility of transient elastography for the diagnosis of fibrosis in pediatric non alcoholic steatohepatitis. *Hepatology* 2008;48:442–8.

71. Joseph AE, Saverymuttu SH, al-Sam S, et al. Comparison of liver histology with ultrasonography in assessing diffuse parenchymal liver disease. *Clin Radiol* 1991;43:26–31.

72. Dasarathy S, Dasarathy J, Khiyami A, et al. Validity of real time ultrasound in the diagnosis of hepatic steatosis: a prospective study. *J Hepatol* 2009;51:1061–7.

73. Saadeh S, Younossi ZM, Remer EM, et al. The utility of radiological imaging in nonalcoholic fatty liver disease. *Gastroenterology* 2002;123:745–50.

74. Shannon A, Alkhouri N, Carter-Kent C, et al. Ultrasonographic quantitative estimation of hepatic steatosis in children with NAFLD. *J Pediatr Gastroenterol Nutr* 2011;53:190–5.

75. Palmentieri B, de Sio I, La Mura V, et al. The role of bright liver echo pattern on ultrasound B-mode examination in the diagnosis of liver steatosis. *Dig Liver Dis* 2006;38:485–9.

76. Mancini M, Prinster A, Annuzzi G, et al. Sonographic hepatic-renal ratio as indicator of hepatic steatosis: comparison with ^{1}H magnetic resonance spectroscopy. *Metabolism* 2009;58:1724–30.

77. Vajro P, Mandato C, Licenziati MR, et al. Effects of *Lactobacillus rhamnosus* strain GG in pediatric obesity-related liver disease. *J Pediatr Gastroenterol Nutr* 2011;52:740–3.

78. Park Sh, Kim Pn, Kim KW, et al. Macrovesicular hepatic steatosis in living liver donors: use of CT for quantitative and qualitative assessment. *Radiology* 2006;239:105–12.

79. Radetti G, Kleon W, Stuefer J, et al. Non-alcoholic fatty liver disease in obese children evaluated by magnetic resonance imaging. *Acta Paediatr* 2006;95:833–7.

80. Pacifico L, Celestre M, Anania C, et al. MRI and ultrasound for hepatic fat quantification: relationships to clinical and metabolic characteristics of pediatric nonalcoholic fatty liver disease. *Acta Paediatr* 2007;96:542–7.

81. Kim SH, Lee JM, Han JK, et al. Hepatic macrosteatosis: predicting appropriateness of liver donation by using MR imaging-correlation with histopathologic findings. *Radiology* 2006;240:116–29.

82. Pozzato C, Verduci E, Scaglioni S, et al. Liver fat change in obese children after a 1-year nutrition-behavior intervention. *J Pediatr Gastroenterol Nutr* 2010;51:331–5.

83. Vajro P, Mandato C, Licenziati MR, et al. Effects of *Lactobacillus rhamnosus* strain GG in pediatric obesity-related liver disease. *J Pediatr Gastroenterol Nutr* 2011;5:740–3.

84. Kim H, Taksali SE, Dufour S, et al. Comparative MR study of hepatic fat quantification using single-voxel proton spectroscopy, two-point Dixon and three-point IDEAL. *Magn Reson Med* 2008;59:521–7.

85. Schwimmer JB, Middleton MS, Deutsch R, et al. A phase 2 clinical trial of metformin as a treatment for non-diabetic paediatric non-alcoholic steatohepatitis. *Aliment Pharmacol Ther* 2005;21:871–9.

86. Bohte AE, van Werven JR, Bipat S, et al. The diagnostic accuracy of US, CT, MRI and 1H-MRS for the evaluation of hepatic steatosis compared with liver biopsy: a meta-analysis. *Eur Radiol* 2011;21:87–97.

87. Castéra L, Vergniol J, Foucher J, et al. Prospective comparison of transient elastography, Fibrotest, APRI, and liver biopsy for the assessment of fibrosis in chronic hepatitis C. *Gastroenterology* 2005;128:343–50.

88. de Lédinghen V, Le Bail B, Rebouissoux L, et al. Liver stiffness measurement in children using FibroScan: feasibility study and comparison with Fibrotest, aspartate transaminase to platelets ratio index, and liver biopsy. *J Pediatr Gastroenterol Nutr* 2007;45:443–50.

89. Fukuzawa Y, Ohashi T, Matsumoto EJ, et al. Efficacy of non-invasive hepatic fibrosis quantification by liver elasticity measurement in NASH—comparison of ultrasonic transient elastography and histo-pathological diagnosis. *Gastroenterology* 2006;130:A-79 [abstract].

90. Francavilla R, Cecinati V, Castellaneta S, et al. Normal values of transient elastography (fibroscan) in children without evidence of liver disease: comparison of adult versus pediatric probe. *Dig Liver Dis* 2009;41S3:202–3 [abstract].

91. Gulizia R, Ferraioli G, Filice C. Open questions in the assessment of liver fibrosis using real-time elastography. *Am J Roentgenol* 2008;190:W370–1.

92. Tilg H, Hotamisligil GS. Nonalcoholic fatty liver disease: cytokine–adipokine interplay and regulation of insulin resistance. *Gastroenterology* 2006;131:934–45.

93. Lebensztejn DM, Wojtkowska M, Skiba E, et al. Serum concentration of adiponectin, leptin and resistin in obese children with non-alcoholic fatty liver disease. *Adv Med Sci* 2009;54:177–82.

94. Nobili V, Manco M, Ciampalini P, et al. Leptin, free leptin index, insulin resistance and liver fibrosis in children with non-alcoholic fatty liver disease. *Eur J Endocrinol* 2006;155:735–43.

95. Manco M, Marcellini M, Giannone G, et al. Correlation of serum TNF-alpha levels and histologic liver injury scores in pediatric nonalcoholic fatty liver disease. *Am J Clin Pathol* 2007;127:954–60.

96. Nobili V, Alkhouri N, Alisi A, et al. Retinol-binding protein 4: a promising circulating marker of liver damage in pediatric non alcoholic fatty liver disease. *Clin Gastroenterol Hepatol* 2009;7:575–9.

97. Alisi A, Manco M, Devito R, et al. Endotoxin and plasminogen activator inhibitor-1 serum levels associated with nonalcoholic steatohepatitis in children. *J Pediatr Gastroenterol Nutr* 2010;50:645–9.

98. Yoneda M, Mawatari H, Fujita K, et al. High-sensitivity C-reactive protein is an independent clinical feature of nonalcoholic steato-hepatitis (NASH) and also of the severity of fibrosis in NASH. *J Gastroenterol* 2007;42:573–82.

99. Adams PC, Passmore L, Chakrabarti S, et al. Liver diseases in the hemochromatosis and iron overload screening study. *Clin Gastroenterol Hepatol* 2006;4:918–23.

100. Nelson JE, Bhattacharya R, Lindor KD, et al. HFE C282Y mutations are associated with advanced hepatic fibrosis in caucasians with nonalcoholic steatohepatitis. *Hepatology* 2007;46:723–9.

101. Reinehr T, Roth CL. Fetuin-A and its relation to metabolic syndrome and fatty liver disease in obese children before and after weight loss. *J Clin Endocrinol Metab* 2008;93:4479–85.

102. Bonnefonnt-Rousselot D, Ratziu V, Giral P, et al. Blood oxidative stress markers are unreliable markers of hepatic steatosis. *Aliment Pharmacol Ther* 2006;23:91–8.

103. Chalasani N, Deeg MA, Crabb DW. Systemic levels of lipid per-oxidation and its metabolic and dietary correlates in patients with nonalcoholic steatohepatitis. *Am J Gastroenterol* 2004;99:1497–502.

104. Bell LN, Molleston JP, Morton MJ, et al. Hepatic lipid peroxidation and cytochrome p-450 2e1 in pediatric nonalcoholic fatty liver disease and its subtypes. *J Clin Gastroenterol* 2011;45:800–7.

105. Piemonte F, Petrini S, Gaeta LM, et al. Protein glutathionylation increases in the liver of patients with non-alcoholic fatty liver disease. *J Gastroenterol Hepatol* 2008;23:e457–64.

106. Nobili V, Parola M, Alisi A, et al. Oxidative stress parameters in paediatric non-alcoholic fatty liver disease. *Int J Mol Med* 2010;26:471–6.

107. Yilmaz J, Kedrah AE, Ozdogan O. Cytokeratin-18 fragments and biomarkers of metabolic syndrome in non alcoholic steatohepatitis. *World J Gastroenterol* 2009;15:4387–91.

108. Vos MB, Barve S, Joshi-Barve S, et al. Cytokeratin 18, a marker of cell death, is increased in children with suspected nonalcoholic fatty liver disease. *J Pediatr Gastroenterol Nutr* 2008;47:481–5.

109. Fitzpatrick E, Mitry RR, Quaglia A, et al. Serum levels of CK18 M30 and leptin are useful predictors of steatohepatitis and fibrosis in paediatric NAFLD. *J Pediatr Gastroenterol Nutr* 2010;51:500–6.

110. Calès P, Lainé F, Boursier J, et al. Comparison of blood tests for liver fibrosis specific or not to NAFLD. *J Hepatol* 2009;50:165–73.

111. Vizzutti F, Arena U, Nobili V. Non-invasive assessment of fibrosis in non-alcoholic fatty liver disease (NAFLD). *Ann Hepatol* 2009;8:89–94.

112. Ratziu V, Massard J, Charlotte F, et al. Diagnostic value of biochemical markers (Fibro Test-Fibro SURE) for the prediction of liver fibrosis in patients with non-alcoholic fatty liver disease. *BMC Gastroenterol* 2006;6:1–14.

113. Shah AG, Lydecker A, Murray K, et al. Comparison of noninvasive markers of fibrosis in patients with non alcoholic fatty liver disease. *Clin Gastroenterol Hepatol* 2009;7:1104–12.

114. Nobili V, Alisi A, Vania A, et al. The pediatric NAFLD fibrosis index: a predictor of liver fibrosis in children with non-alcoholic fatty liver disease. *BMC Med* 2009;7:21.

115. Nobili V, Parkes J, Bottazzo G, et al. Performance of ELF serum markers in predicting fibrosis stage in pediatric non-alcoholic fatty liver disease. *Gastroenterology* 2009;136:160–7.

116. Alkhouri N, Carter-Kent C, Lopez R, et al. A combination of the pediatric NAFLD fibrosis index and enhanced liver fibrosis test identifies children with fibrosis. *Clin Gastroenterol Hepatol* 2011; 9:150–5.

117. Vajro P, De Chiara C, D'Armiento M, et al. Serum hyaluronic acid and laminin in children with chronic hepatitis B. *Eur J Gastroenterol Hepatol* 1994;6:1023–6.

118. Nobili V, Alisi A, Torre G, et al. Hyaluronic acid predicts hepatic fibrosis in children with non-alcoholic fatty liver disease. *Transl Res* 2010;156:229–34.

119. Miele L, Forgione A, La Torre G, et al. Serum levels of hyaluronic acid and tissue inhibitor of metalloproteinase-1 combined with age predict the presence of nonalcoholic steatohepatitis in a pilot cohort of subjects with nonalcoholic fatty liver disease. *Transl Res* 2009; 154:194–201.

120. Poynard T, Ratziu V, Naveau S, et al. The diagnostic value of biomarkers (SteatoTest) for the prediction of liver steatosis. *Comp Hepatol* 2005;4:10.

121. Poynard T, Ratziu V, Charlotte F, et al. Diagnostic value of biochemical markers (NashTest) for the prediction of non alcoholosteato hepatitis in patients with non-alcoholic fatty liver disease. *BMC Gastroenterol* 2006;6:1–16.

122. Munteanu M, Ratziu V, Morra R, et al. Noninvasive biomarkers for the screening of fibrosis, steatosis and steatohepatitis in patients with metabolic risk factors: FibroTest-FibroMax experience. *J Gastrointest Liver Dis* 2008;17:187–91.

123. Trak-Smayra V, Dargere D, Noun R, et al. Serum proteomic profiling of obese patients: correlation with liver pathology and evolution after bariatric surgery. *Gut* 2009;58:825–32.

124. Bell LN, Theodorakis JL, Vuppalanchi R, et al. Serum proteomics and biomarker discovery across the spectrum of non-alcoholic fatty liver disease. *Hepatology* 2010;51:111–20.

125. Loria P, Adinolfi LE, Bellentani S, et al. Practice guidelines for the diagnosis and management of nonalcoholic fatty liver disease. A decalogue from the Italian Association for the Study of the Liver (AISF) Expert Committee. *Dig Liver Dis* 2010;4:272–82.

126. Ratziu V, Bellentani S, Cortez-Pinto H, et al. A position statement on NAFLD/NASH based on the EASL 2009 special conference. *J Hepatol* 2010;2:372–84.

127. Vajro P, Veropalumbo C. Citrin deficiency: learn more and don't forget to add it to the list of neonatal cholestasis and the NASH trash bin. *J Pediatr Gastroenterol Nutr* 2010;50:578–9.