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EDITORIAL

Controversy in the diagnosis of pediatric non-alcoholic fatty liver disease

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Abstract

In the last years childhood obesity has reached epidemic diffusion with about 200 million school-age children worldwide being overweight or obese. Simultaneously, also the prevalence of obesity comorbidities has been increased and the non-alcoholic fatty liver disease (NAFLD) has become the most common form of liver disease in childhood. Also if there are some not-invasive diagnostic possibilities, the diagnostic gold standard is represented by hepatic biopsy giving to the clinicians the possibility to both diagnose the NAFLD and evaluate its progression to fibrosis or cirrhosis with greater certainty than other techniques. The use of liver biopsy in clinical practice causes debate among health care providers. Most patients with NAFLD have a good prognosis and, therefore, the risks of a liver biopsy seem to outweigh the clinical benefits. It represents an impractical screening procedure because it is both expensive and invasive and, moreover, sampling error of liver biopsy can result in substantial misdiagnosis and staging inaccuracies because histological lesions of non-alcoholic steatohepatitis are unevenly distributed throughout the liver parenchyma. The liver biopsy limitations have led the clinicians to use, also if highly imperfect, non-invasive methods to diagnose and stage NAFLD. In this editorial the main diagnostic controversies in pediatric NAFLD are examined.

Key words: Pediatric; Non-alcoholic fatty liver disease; Biopsy; Liver; Ultrasound; Alanine transaminase

Core tip: Because of the rising obesity prevalence, the non-alcoholic fatty liver disease (NAFLD) has become the most common form of liver disease in childhood. The diagnostic gold standard is represented by hepatic biopsy but the use of liver biopsy in clinical practice causes debate among health care providers because most patients with NAFLD have a good prognosis and, therefore, the risks of a liver biopsy seem to outweigh the clinical benefits. The liver biopsy limitations have led the clinicians to use non-invasive methods to diagnose and stage NAFLD. In this editorial the main diagnostic controversies in pediatric NAFLD are examined.

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INTRODUCTION

The pediatric obesity prevalence has been constantly increasing in the last years[1]. This rising has lead to an increased incidence of obesity comorbidities, including non-alcoholic fatty liver disease (NAFLD)[2]. NAFLD, in fact, is the most common pediatric liver disease with a prevalence more than doubled in the last decades[3]. Between 3% and 11% of the pediatric population present NAFLD[4,5], with highest prevalence (46%) reached among overweight and obese children and adolescents[6].

NAFLD comprehends conditions ranging from fatty liver or steatohepatitis, to cirrhosis and its complications (*e.g.*, hepatocellular carcinoma and portal hypertension)[7,8]. Hepatic biopsy represents the diagnostic gold standard defining the NAFLD when more than 5% of hepatocytes present fat infiltration in absence of other demonstrable liver diseases[7]. The NAFLD progression to advanced fibrosis and cirrhosis is a long-term phenomenon and then, NAFLD complications present low incidence in children. A recent pediatric autopsy series demonstrated that the non-alcoholic steatohepatitis (NASH) prevalence was 2.96%. Moreover, NASH was shown in about 23% of children with NAFLD and bridging fibrosis and/or cirrhosis were present in 9% of children with NASH[6].

Therefore, considering the low prevalence of NASH in children could be reasonable to use non-invasive methods to evaluate hepatic steatosis in childhood obesity, reserving the liver biopsy only for specific indications as shown below. The use of liver biopsy in daily clinical practice causes debate among pediatricians because in childhood the NAFLD presents a good prognosis and therefore, the liver biopsy related risks could outweigh the clinical benefits. Moreover, the liver biopsy is not practicable as screening methods because it is both expensive and invasive[9]. However, considering the increased NAFLD prevalence, it is more likely to evaluate a child with NAFLD determined by a primary liver disease (*e.g.*, autoimmune liver disease or Wilson disease) and therefore it is fundamental to not miss a treatable condition[9].

Liver biopsy is the gold standard technique giving the possibility to differentiate NAFLD from NASH[10] but the clinicians must be aware of the possibility that sampling error of liver biopsy can determine misdiagnosis and staging imprecisions because histological NASH lesions are not uniformly distributed throughout the hepatic parenchyma[11].

WHEN TO PERFORM A LIVER BIOPSY?

Although there are other diagnostic possibilities, at the present time liver biopsy still represents the only way for a definitive pediatric NAFLD diagnosis.

The principal aims of the liver biopsy examining a child with the suspect of NAFLD are to confirm or exclude the diagnosis, to evaluate if other concomitant liver diseases are present and to quantify the degree of liver involvement[12].

Being invasive, liver biopsy should be considered for children who will "benefit" of this exam and, therefore, the ESPGHAN Hepatology Committee indicated the following criteria to perform a liver biopsy when suspecting NAFLD. They are: (1) the exclusion of other treatable disease; (2) the suspect of advanced liver disease; (3) before pharmacological/surgical treatment; and (4) as part of a structured intervention protocol or clinical research trial. The Committee also recommended to perform the liver biopsy for differential diagnosis only if other non-invasive investigations have not been conclusive[9].

Also if the liver biopsy is the gold standard for the NAFLD diagnosis and for the evaluation of the progression to NASH, it presents limitations. Even if liver biopsy is performed under ultrasound guidance, it is invasive and could lead to complications represented by pain, bleedings, organ perforation (more likely if the biopsy is performed in blind) and death (0.01% of the cases)[12]. Another limitation is the operator experience in performing biopsy[13]. In fact, if the samples size in not appropriate the diagnostic accuracy could be lower[11,14]. Also the NAFLD itself could limit the biopsy diagnostic accuracy, in fact it could not present homogeneous fibrosis diffusion and then the histopathological samples could not show these areas. Apart of the experience in performing the biopsy, it is also important the experience of the pathologist[13]. Kleiner *et al*[15] demonstrated that pathologists agreements about steatosis, ballooning and fibrosis were good but not so strong for location of steatosis and for inflammation[16].

WHAT ARE THE ALTERNATIVES TO A LIVER BIOPSY?

In daily clinical practice the observation of an overweight/obese child with elevated serum ALT and γ-glutamyl transpeptidase (GGT) levels and/or ultrasound (US) evidence of bright liver suggests to the clinicians the NAFLD diagnosis[9]. The ALT levels could be in the normal range also if NAFLD is present and then the sensitivity of this marker is low[17]. Moreover there is evidence that degree of ALT elevation is not useful to predict the presence[18,19] or severity of NAFLD histological findings[20]. In fact, advanced fibrosis could be present without ALT levels elevation[17,21]. However, being the ALT dosage inexpensive and easily available,

it could be considered as useful test for the screening and initial evaluation of the NAFLD[9]. Evaluating ALT levels it is important to use age and sex specific centiles to not have misleading interpretations of these values[22]. Could be reasonable to use as cut-off ALT levels of 60 U/L and 40 U/L in children aged less than 18 mo and more than 18 mo, respectively[22,23]. As marker of advanced fibrosis in NAFLD, GGT could be evaluated[24]. Moreover, there is evidence that serum ferritin levels higher than 1.5 upper normal limits are associated with hepatic iron deposition, NASH diagnosis, and advanced hepatic fibrosis in NAFLD affected patients[25].

As imaging technique, the US is useful in NAFLD screening because of lack of invasiveness, widely diffusion and being inexpensive[26]. US give also the possibility to identify any evidence of portal hypertension[27]. The liver at the US scan appears similar to other solid organs. When the liver presents brightness or hyperechogenicity compared with the right kidney or spleen, a diagnosis of steatosis could be performed. The degree of fatty infiltration could be quantified by the echogenicity degree[9]. Steatosis is detectable by US when fat infiltration affects more than 20% of hepatocytes[28] and the sensitivity and specificity are 100% and 90% respectively[29]. However, around the US use in pediatric NAFLD there is an important debate. Recently, Awai *et al*[30] concluded their systematic review affirming that available evidence does not support the use of ultrasonography for the diagnosis or grading of fatty liver in children. Therefore, also if US can be used as screening because of its relative low cost, large diffusion in medical community and feasibility in the pediatric population[31], the clinicians must be aware of the US limits: this method can not differentiate between steatosis and steatohepatitis, can not establish with certainty the degree of fatty infiltration, can not differentiate between steatosis and other diffuse liver diseases characterized by increased echogenicity, and finally, can not always identify the spared or focal steatosis areas from hepatic focal lesions[32]. In future, using the computerized analysis of US images and especially the new elastographic techniques the US capacity to individuate steatosis could increase[32].

Unenhanced computed tomography (CT) presents more specificity than US for the fatty liver quantification[9]. With this technique the fatty liver is detected measuring the difference in liver and spleen attenuation values with a sensitivity and specificity in adults compared with liver biopsy of 82% and 100%, respectively[33]. However, there is evidence of a not clinically acceptable CT diagnostic performance for quantitative assessment of macrovesicular steatosis[33]. Moreover, considering that repeated CT carries an established risk of increase incidence of cancer in the pediatric patients[34], the CT use in pediatric NAFLD is to consider impracticable and should be reserved only for emergencies and malignancies.

Magnetic resonance (MRI) could be of interest in childhood because neither invasive nor irradiating and presents the capacity to reliably quantify liver fat infiltration[9]. Pacifico *et al*[35] demonstrated a good correlation between MRI and US especially in pediatric patients with moderate and severe steatosis. Data about sensitivity and specificity of MRI compared to liver biopsy in childhood are not available; in adult population, the sensitivity and specificity compared to histopathological findings are 100% and 90.4%, respectively[36]. However, because histology gives a fraction of hepatocytes involved rather than fraction of tissue volume occupied by fat as available with MRI, it not possible to perform a direct comparison. Also if this technique is not invasive and without radiations, it is expensive and not widely available. In addition, young and not collaborative children performing MRI need of sedation. Therefore, at the present time, MRI is not applicable for a screening program and the available data are insufficient to give evidence-based recommendations regarding its use in children[30].

^{1}H-MR spectroscopy (^{1}H-MRS) evaluates the hepatic triglyceride content by directly measuring protons in the acyl groups of the liver tissue triglycerides and then can quantify the hepatic steatosis. It has been successfully used to measure hepatic fat content in a pediatric cohort of histopathological assessed NASH before and after pharmacological treatment[37]. This technique is time-consuming and requires off-scan analysis by an expert. Therefore, at present time, its use is limited to research studies and not suitable for daily clinical use[9].

Recently, the transient elastography has been introduced and it showed the capacity to evaluate liver fibrosis based on stiffness being noninvasive, rapid, painless, and reproducible[9]. There is a good correlation with hepatic histology both in adults[38] and children[39] with chronic liver disease including NAFLD. This technique is not yet performed in everyday clinical practice also if a probe with size appropriate also for smaller children has been made[40].

A recent method for non-invasive steatosis assessment is the controlled attenuation parameter (CAP) evaluated with transient elastography. It can quantify hepatic fat content along with assessment of liver stiffness. The available data, however, are limited to adult population and to the probes M and XL. If will be available similar data in pediatric population, CAP should become an important tool for liver fat content quantification in children[41].

The magnetic resonance elastography (MRE) uses a modified phase-contrast MRI sequence to visualize propagating share waves in tissues. It could be used in addition to MRS to evaluate the degree of steatosis and of liver stiffness in a not invasive way; however, further studies are needed before introducing MRE in the daily use[9].

An alternative elastography method is represented

Table 1 Advantages and disadvantages of non-alcoholic fatty liver disease diagnostic techniques

Diagnostic techniques	Advantages	Disadvantages
Biopsy	Gold Standard, highest sensitivity and specificity	Invasive and expensive; Sampling error of liver biopsy can result in substantial misdiagnosis and staging inaccuracies;
ALT serum levels dosage	Widely available and inexpensive, useful as screening	Low sensitivity
Ultrasound	Widely available, inexpensive. When the percentage of steatosis is > 20%, sensitivity and specificity are acceptable. Useful as screening	Can not differentiate between steatosis and steatohepatitis; Can not establish with certainty the degree of fatty infiltration; Can not differentiate between steatosis and other diffuse liver diseases characterized by increased echogenicity; Can not always identify the spared or focal steatosis areas from hepatic focal lesions
Unenhanced computed tomography	Good sensitivity and specificity compared to live biopsy	Quantitative assessment of macrovesicular steatosis is not clinically acceptable; Radiations exposure
Magnetic resonance	Good sensitivity and specificity compared to live biopsy in adult population It is not invasive and it is not irradiating	There are insufficient data to make evidence-based recommendations regarding its use in children
^{1}H-MR spectroscopy	It has been applied successfully in a pediatric pilot study to measure hepatic fat content in patients with histopathological evidence of NASH before and after pharmacological treatment	It is time-consuming and requires off-scan analysis by an expert Its use appear to be more appropriate for research studies and not suitable for widespread use
Transient elastography	Good correlation with hepatic histology both in adults and children	It is not yet performed in everyday clinical practice
Magnetic resonance elastography	It could be a complement to ^{1}H-MR spectroscopy to estimate non-invasively the degree of steatosis and degree of liver stiffness	Further studies are needed before to validate this method
Biomarkers, prediction scores Equations and tests	Could help the clinicians to perform NAFLD diagnosis and evaluate progression to NASH without performing liver biopsy	There are contrasting results and more validation studies are needed

NAFLD: Non-alcoholic fatty liver disease; ALT: Alanine transaminase; NASH: Non-alcoholic steatohepatitis; ^{1}H-MR: ^{1}H-Magnetic resonance.

by the Acoustic Radiation Force Impulse Imaging (ARFI), a new non-invasive method quantifying the tissue stiffness during ultrasound examination. Data in pediatric age are available but further studies are needed to validate this technique[42].

In literature many studies evaluating biomarkers of hepatic inflammation[43-48], oxidative stress[49-53], apoptosis[54-56], or hepatic fibrosis[57,58] , with the aim to identify children with NAFLD and predict those at increased risk for progression to NASH, are available. Moreover, also prediction scores equations[59,60] or tests containing combinations of markers (such as Steatotest[61] or NashTest[58]) have been developed with contrasting results. Noninvasive prediction scores for NAFLD are highly demanded to enable clinicians to screen for NAFLD easily and rapidly[59]. Particularly, accurate exclusion of the presence of NAFLD in high-risk groups would allow clinicians to select patients needing more expensive and invasive diagnostics[59]. Koot *et al*[59] built a new equation (ALT-HOMA-leptin) that performed better than the equations previously built (NAFLD liver fat score, fatty liver index, hepatic steatosis index and ped-NAFLD score) but its diagnostic accuracy for excluding NAFLD was still insufficient for daily clinical practice. Also prediction scores evaluating advanced fibrosis in children with NAFLD have been tested. Alkhouri *et al*[60] showed that noninvasive hepatic fibrosis scores developed in adults have poor performance in diagnosing advanced fibrosis in children with NAFLD. Moreover, in this study, a new pediatric specific score called PNFS was proposed and showed improved performance characteristics[60]. However, the authors stated that the score must be validated in other populations before it can be recommended for daily clinical practice in diagnosing advanced hepatic fibrosis in children with NAFLD[60].

In conclusion, these are interesting research fields but, before biomarkers, prediction scores or tests could be used in clinical practice more validation studies are needed.

CONCLUSION

The increasing prevalence of pediatric NAFLD and the risks, limitations and costs of liver biopsy have led the clinicians to use, also if highly imperfect, non-invasive methods to diagnose and stage NAFLD (these methods are summarized in Table 1). Considering that, in pediatric age, the NAFLD is, frequently, a benign condition, we think that the most important diagnostic issue for the clinicians is to not perform a certain NAFLD diagnosis but to not miss other causes underlying or mimicking the NAFLD. Because of the high prevalence of NAFLD in obese children and adolescents[6], we suggest, on the basis of available literature, to perform ALT dosage and hepatic ultrasound as screening of NAFLD in this population. However when there are the conditions

indicated by the ESPGHAN Hepatology Committee is reasonable to perform the liver biopsy referring the patient to a specialist centre with good experience both in performing the biopsy and in evaluating the histopathological lesions. The Latin aphorism "primum non nocere" must drive our clinical practice.

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