

Original Article

Diagnostic performance of ultrasound elastography for assessing liver stiffness in children with non-alcoholic fatty liver disease and biliary atresia

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Abstract: Purpose: To evaluate the diagnostic performance of two-dimensional shear wave elastography (2D-SWE) in assessing liver stiffness in pediatric patients with NAFLD and in infants with biliary atresia (BA). Methods: In this retrospective study with case-control sampling, 129 children with NAFLD and 129 healthy controls, as well as 83 infants with BA and 83 age-matched infants with non-BA cholestasis, were enrolled. Liver stiffness measurement (LSM) was performed using 2D-SWE. Demographic, anthropometric, biochemical, and conventional ultrasound data were collected at enrollment. Results: In the NAFLD cohort, LSM was significantly higher in the NAFLD group than in healthy controls (4.42 ± 0.92 vs. 3.74 ± 0.28 kPa; $P < 0.001$), with an area under the curve (AUC) of 0.865 (95% CI: 0.820-0.910). All other metabolic, inflammatory, and ultrasound measurements also showed significant differences between the two groups (all $P < 0.05$), except for age, sex, total bilirubin, and direct bilirubin ($P > 0.05$). In the BA cohort, LSM was significantly higher in BA infants than in non-BA cholestasis infants (13.58 ± 8.46 vs. 6.31 ± 1.02 kPa; $P < 0.001$; AUC = 0.890, 95% CI: 0.865-0.935). BA infants also showed significantly elevated liver enzymes, bilirubin, inflammatory markers, and rates of gallbladder abnormality, splenomegaly, and ascites, as well as lower lipid levels (all $P < 0.05$). Conclusion: 2D-SWE provides good diagnostic accuracy for detecting liver stiffness in pediatric NAFLD and BA, supporting its use as a non-invasive assessment tool for group discrimination in these two distinct pediatric liver diseases.

Keywords: Pediatric liver disease, shear wave elastography, non-alcoholic fatty liver disease, biliary atresia, liver stiffness measurement

Introduction

Non-alcoholic fatty liver disease (NAFLD) - also called MASLD (Metabolic Dysfunction-Associated Steatotic Liver Disease) - is now widely recognized as a major pediatric health issue, reflecting the worldwide increase in childhood obesity [1, 2]. NAFLD covers a broad range of disease states, from simple steatosis to non-alcoholic steatohepatitis (NASH), potentially advancing to fibrosis and cirrhosis. The underlying causes of pediatric NAFLD are multifactorial, including genetic predisposition, insulin resistance, and metabolic dysregulation. These factors contribute to hepatic fat accumulation, leading to inflammation and fibrosis [3, 4]. Early identification and manage-

ment are crucial for preventing progression to advanced liver disease. However, diagnosing NAFLD remains challenging because it is often asymptomatic. Traditional diagnostic methods such as liver biopsy are invasive and not suitable for routine screening in pediatric populations.

Biliary atresia (BA) is another critical pediatric hepatic disorder marked by advancing bile duct damage, resulting in obstructive cholestasis and subsequent liver fibrosis. It is the most common indication for liver transplantation in children. BA typically presents within the first few months of life, often with jaundice persisting beyond the neonatal period [5, 6]. Early diagnosis and surgical intervention, such as

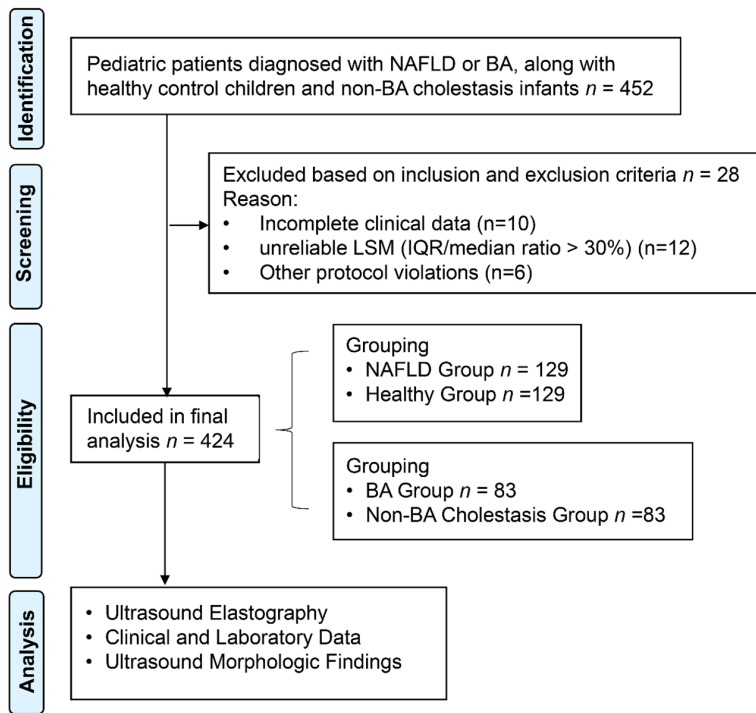


Figure 1. Participant flow diagram. Healthy controls were frequency-matched to the NAFLD group by age (± 1 year) and sex; infants with non-BA cholestasis were age- and sex-matched to the BA group. The two radiologists performing 2D-SWE were blinded to participants' diagnostic status at the time of image acquisition and measurement. LSM: liver stiffness measurement; IQR: interquartile range; NAFLD: nonalcoholic fatty liver disease; BA: biliary atresia; kPa: kilopascals.

Kasai portoenterostomy, are essential to improve survival and delay the need for liver transplantation. However, distinguishing BA from other causes of neonatal cholestasis can be challenging, particularly in the early stages when clinical symptoms overlap. Diagnostic delays can lead to irreversible liver damage and poor outcome [7, 8].

Thus, there is an urgent need for reliable noninvasive diagnostic methods to differentiate NAFLD and BA from others early and assess liver stiffness to guide timely therapeutic intervention. Ultrasound elastography has emerged as a promising non-invasive technique for evaluating liver stiffness. This imaging method measures the mechanical properties of liver tissue, providing valuable information about the degree of fibrosis. Unlike traditional ultrasound, which assesses primarily liver morphology, elastography can quantify liver stiffness, offering insight into the structural changes

associated with various liver diseases [9-11]. Its application in pediatric settings, particularly for distinguishing among different pediatric liver diseases, remains under-explored. Given the unique pathophysiology of pediatric liver diseases, it is essential to evaluate the diagnostic accuracy and clinical relevance of ultrasound elastography in this population.

The ability of ultrasound elastography to assess liver stiffness in children with NAFLD and BA could significantly affect clinical practice. The non-invasive nature of elastography makes it particularly suitable for pediatric use, reducing the need for more invasive procedures like liver biopsy. This study aimed to explore the clinical significance of ultrasound elastography in assessing liver stiffness in pediatric patients with NAFLD and BA. Through systematic evaluation, we as-

essed the diagnostic utility of ultrasound elastography and its potential role in clinical evaluation.

Materials and methods

Patient selection

This retrospective study with case-control sampling was conducted at Affiliated Children's Hospital of Jiangnan University (Wuxi Children's Hospital). Consecutive pediatric patients diagnosed with NAFLD or BA, along with healthy control children and non-BA cholestasis infants, were identified from the hospital's electronic medical records between January 2018 and December 2025. A participant inclusion flow diagram is provided in **Figure 1**.

Healthy controls were frequency-matched to the NAFLD group by age (± 1 year) and sex. The two radiologists performing 2D-SWE were blinded to the patients' final diagnoses at the

time of image acquisition and measurement. The clinicians who established the reference diagnoses (NAFLD, BA, or control status) were independent of the elastography operators and were blinded to LSM results. For conventional ultrasound interpretation, the two radiologists were also blinded to the patients' clinical and laboratory data at the time of image review; disagreement was resolved by consensus after reviewing the images and clinical data. No failed elastography examinations occurred among the included participants, since all measurements met the predefined quality criteria.

The study protocol was approved by the ethics committee of Affiliated Children's Hospital of Jiangnan University (Approval No. WXCH2026-04-92). Given that the study adopted a retrospective design and used anonymized patient records, the requirement for written informed consent was waived by the ethics committee.

A total of 424 children were included and divided into two independent cohorts. The first cohort (the NAFLD cohort) comprised 129 children with NAFLD and 129 matched healthy children. The second cohort (the BA cohort) comprised 83 infants with BA and 83 age- and sex-matched infants with non-BA cholestasis. Healthy controls were selected from children who underwent routine health check-ups at the same institution during the same period; they were frequency-matched to the NAFLD group by age (± 1 year) and sex. The two radiologists performing 2D-SWE were blinded to the patients' final diagnoses (NAFLD or BA versus controls) at the time of image acquisition and measurement.

Participant selection standards

NAFLD cohort: The NAFLD group included children aged 6-18 years who were diagnosed with NAFLD in the absence of other causes of chronic liver disease, including chronic viral infections (HBV/HCV), autoimmune liver disease, Wilson disorder, AAT deficiency, or medication-induced hepatic injury. In addition, each patient was required to have one or more metabolic risk indicators, defined as overweight or obesity (body mass index $\geq$ 85th percentile for age and sex), dyslipidemia (total cholesterol $\geq$ 5.18 mmol/L or triglycerides $\geq$ 1.70 mmol/L or high-density lipoprotein cholesterol $\leq$ 1.03 mmol/L), or impaired fasting glucose (fasting blood glu-

cose $\geq$ 5.6 mmol/L), which served as a proxy for metabolic risk in the absence of validated insulin-resistance indices. The diagnosis of NAFLD was made in accordance with the NASPGHAN clinical practice standards [12].

The diagnosis of NAFLD was based on a composite clinical algorithm that did not require ultrasonographic evidence of steatosis as an absolute prerequisite. Specifically, a diagnosis could be established in the presence of at least one cardiometabolic risk factor, elevated ALT (≥ 40 U/L on at least two separate occasions at least one month apart), and after exclusion of other liver diseases, even in the absence of sonographic steatosis, given that gray-scale ultrasound has limited sensitivity for mild steatosis (i.e., $< 20\%$ hepatic fat content). This composite algorithm had been predefined in the institutional clinical protocol for pediatric NAFLD diagnosis before the start of data extraction and was applied prospectively during routine clinical care.

The healthy control group consisted of children who underwent routine health check-ups at the same institution during the same period, with no known chronic diseases, normal hepatic enzyme panels (ALT ≤ 40 U/L, AST ≤ 40 U/L, total bilirubin within normal age-specific ranges), and no evidence of hepatic steatosis on abdominal ultrasound.

Exclusion criteria for both the NAFLD group and the healthy control group included coexisting liver diseases, significant systemic illnesses affecting liver function, incomplete clinical or elastographic data, and any condition that would confound the interpretation of liver stiffness measurements.

Exclusion of other liver diseases was based on negative serological markers for hepatitis B (HBsAg) and hepatitis C (anti-HCV), normal serum ceruloplasmin and 24-hour urinary copper excretion for Wilson's disease, normal alpha-1 antitrypsin levels and phenotype, and absence of a history of hepatotoxic drug exposure. Autoimmune hepatitis was excluded by negative ANA, anti-SMA, and anti-LKM-1 antibodies, with normal immunoglobulin G levels. Discordant clinical, biochemical, and ultrasound findings were adjudicated by consensus between two senior pediatric hepatologists who reviewed all available data independently.

BA cohort inclusion and exclusion criteria: The BA group included infants aged 0-12 months with a confirmed diagnosis of BA. Of the 83 BA infants, 69 (83.1%) were diagnosed by intraoperative cholangiography showing no patent extrahepatic bile ducts. The remaining 14 cases (16.9%) were initially diagnosed by liver biopsy showing bile duct proliferation, portal tract edema, and portal fibrosis; all 14 subsequently underwent laparotomy with intraoperative cholangiography, which confirmed the absence of patent extrahepatic bile ducts in every case. Thus, intraoperative cholangiography ultimately served as the definitive reference standard for all 83 BA cases. In the non-BA cholestasis group (n = 83), diagnoses were confirmed by the following specific methods: 38 infants had idiopathic neonatal hepatitis, confirmed by liver biopsy showing giant cell transformation and lobular disarray in the absence of bile duct obstruction; 21 had PFIC, confirmed by genetic testing (pathogenic variants in ATP8B1, ABCB11, or ABCB4) in 15 cases and by liver biopsy showing characteristic canalicular cholestasis and giant cell hepatitis in the remaining 6 cases; 14 had Alagille syndrome, confirmed by genetic testing (JAG1 or NOTCH2 pathogenic variants) in 10 cases and by characteristic clinical findings (paucity of interlobular bile ducts on biopsy, pulmonary stenosis, and butterfly vertebrae) in the remaining 4 cases; and 10 had choledochal cyst, confirmed by intraoperative cholangiography demonstrating a dilated extrahepatic bile duct without biliary atresia. All non-BA diagnoses were established using the respective standard diagnostic modalities. All BA patients were assessed before the Kasai portoenterostomy. The non-BA cholestasis group included age-matched infants with neonatal cholestasis due to other reasons, which had been ruled out by appropriate diagnostic tests, such as idiopathic neonatal hepatitis, PFIC, Alagille syndrome, and choledochal cyst. Exclusion criteria for both the BA group and the non-BA cholestasis group included coexisting major congenital anomalies affecting liver function, incomplete clinical or elastographic data, post-Kasai status, and any condition that would compromise the reliability of liver stiffness measurements.

Matching was frequency-based (rather than individual matching), with a 1:1 selection ratio for both comparisons. For the NAFLD cohort, controls were matched to cases by age (± 1

year) and sex. For the BA cohort, controls were matched to BA cases by age (± 2 months) and sex. The matching variables and tolerances were predefined before case selection. Non-disease-related exclusion criteria-incomplete clinical or elastographic data, unreliable LSM (IQR/median $> 30\%$), and protocol violations-were applied identically across all four groups.

Ultrasound elastography

All patients underwent 2D-SWE using a standardized protocol on the PHILIPS EPIQ7 ultrasound system (Philips Healthcare, Bothell, WA, USA) equipped with a C5-1 convex array transducer (1-5 MHz) for pediatric patients and an eL18-4 linear array transducer (4-18 MHz) for infants. For the NAFLD cohort and the healthy control cohort, examinations were performed after fasting for at least 4 hours. For the BA cohort and the non-BA cholestasis cohort, examinations were performed within 1 week prior to Kasai portoenterostomy or diagnostic workup after 4 hours of fasting. With the patient in the supine position and the right maximally abducted to widen the intercostal space, the transducer was positioned in the right intercostal space over the right hepatic lobe, targeting segments V and VIII. Patients were instructed to breathe shallowly and to hold their breath briefly during each acquisition to minimize motion artifacts and breathing-induced variations in liver stiffness. For uncooperative infants, examinations were attempted during natural sleep or after feeding; when necessary, oral chloral hydrate (50 mg/kg) was administered under the supervision of a pediatrician to achieve sedation, ensuring measurement quality.

A circular region of interest (ROI) measuring approximately 1.0-2.0 cm in diameter was placed in the parenchyma of the right hepatic lobe at a depth of 2-5 cm from the liver capsule. For children in the NAFLD cohort and healthy controls, the ROI size was standardized to 1.5-2.0 cm with a measurement depth of 3-5 cm. For infants in the BA and non-BA cholestasis cohorts, the ROI size was standardized to 1.0-1.5 cm with a measurement depth of 2-4 cm. Care was taken to avoid large vessels, the gallbladder, the hepatic capsule, and focal liver lesions. The ROI was manually positioned within a homogeneous area of the liver parenchyma that appeared free of large vessels and artifacts on the B-mode image. For each

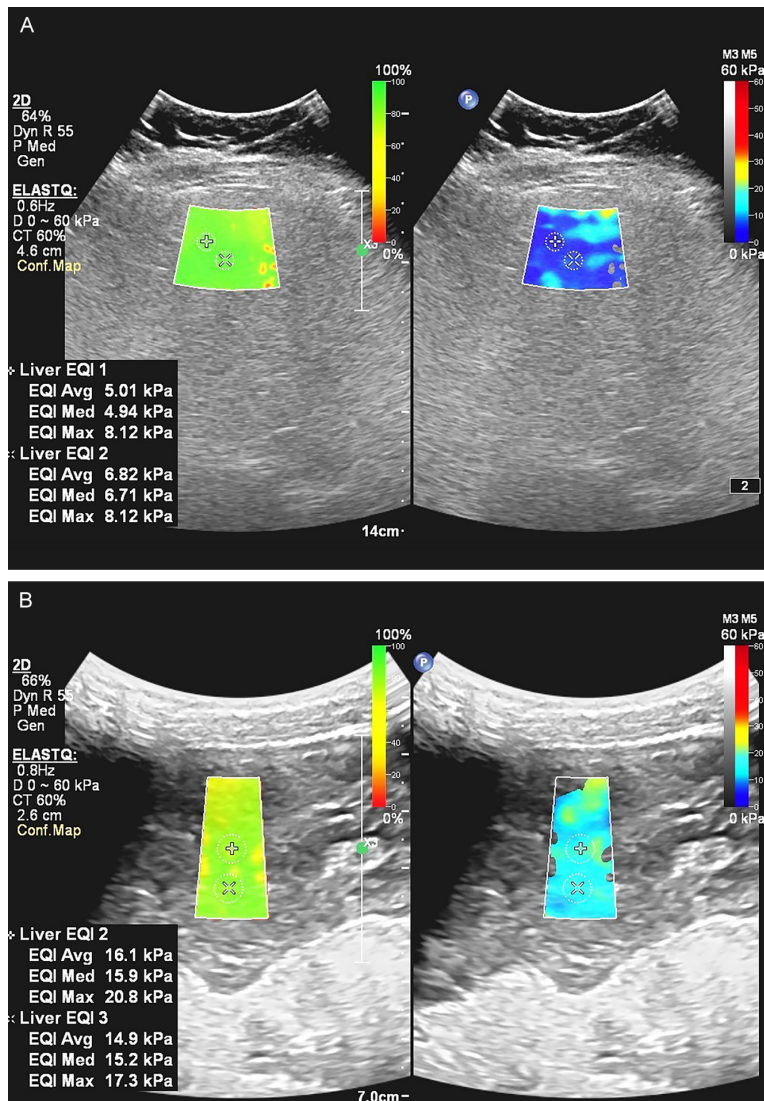


Figure 2. Representative 2D-SWE images of liver stiffness in (A) a child with NAFLD and (B) an infant with biliary atresia.

patient, ten valid measurements were obtained consecutively under the same respiratory condition. Each measurement was acquired only when the quality map within the ROI showed uniform filling, indicating reliable shear wave propagation. For the EPIQ7 system, the manufacturer's recommended quality criterion (shear wave propagation map fill rate > 95%) was applied to ensure measurement validity. The median value was used for analysis. Examinations with an IQR/median ratio exceeding 30% were considered unreliable and were excluded from the analysis. Liver stiffness measurement (LSM) was expressed in kPa. All examinations were performed by two experi-

enced pediatric radiologists (> 5 years of elastography experience). Operator allocation was consistent across the study: one radiologist performed all examinations for the NAFLD cohort (children) and the other performed all examinations for the BA cohort (infants), ensuring consistency within each cohort. Fasting status was maintained for at least 4 hours in all selected participants; in cases where feeding was necessary to achieve natural sleep, the examination was performed at least 2 hours after feeding to minimize postprandial effects. When infants remained uncooperative, oral chloral hydrate (50 mg/kg) was administered under pediatrician supervision to achieve sedation. Interobserver agreement was assessed using the ICC based on 20 randomly selected cases (10 from each cohort) that were measured independently by both operators. Interobserver reliability was quantified by a two-way random-effects, absolute-agreement ICC, with two replicate measurements per case. The overall ICC was 0.91 (95% CI: 0.86-0.95), with separate ICCs of 0.92 (95% CI: 0.87-

0.95) for the NAFLD cohort and 0.89 (95% CI: 0.83-0.93) for the BA cohort, indicating excellent interobserver reproducibility. The reliability subset was selected by simple random sampling from each cohort using a random-number generator. Among the 83 infants in the BA cohort and 83 in the non-BA cholestasis group, 28 infants (16.9%) were examined after feeding (at least 2 hours post-feed) to achieve natural sleep, and 19 infants (11.4%) required oral chloral hydrate sedation (50 mg/kg) under pediatrician supervision. No sedation-related adverse events occurred. Representative 2D-SWE images of a child with NAFLD and an infant with biliary atresia are shown in **Figure 2**.

Clinical and laboratory data

Patient demographics, physical metrics, and clinical findings were obtained from the EMR system for all participants. For the NAFLD cohort and the healthy control cohort, the following demographic and clinical findings were recorded: age (years), sex, BMI, waist circumference, and systolic blood pressure. For the BA cohort and the non-BA cholestasis cohort, recorded parameters included age, sex, body weight, body length, and age at onset of jaundice (days). Age at onset of jaundice was defined as the age (in days) when jaundice was first noted by the parent or caregiver.

Biochemical assays were performed on venous specimens drawn within one week prior to the elastography examination. ALT, AST, GGT, ALP, TBil, and DBil were measured using clinical chemistry analyzer (AU5800, Beckman Coulter, Inc., Brea, CA, USA) with manufacturer-provided reagents. TC, TG, HDL-C, and LDL-C, fasting venous plasma glucose and CRP were analyzed using the same Beckman Coulter AU5800 analyzer. ESR was measured using the Westergren method in whole blood collected in sodium citrate tubes.

Ultrasound morphologic findings

All patients underwent conventional B-mode abdominal ultrasound examination using the same ultrasound system (PHILIPS EPIQ7) prior to elastography. Increased hepatic echogenicity (steatosis) was defined as diffuse increase in liver echogenicity relative to the renal cortex on the same image, accompanied by far-field attenuation and loss of definition of the diaphragm and hepatic vessel walls. Steatosis was graded subjectively by the same two pediatric radiologists as mild (slight increase in echogenicity with clear visualization of diaphragm and intrahepatic vessels), moderate (moderate increase with slight attenuation and blurring of vessel walls), or severe (marked increase with deep attenuation and poor diaphragm definition); any degree of increased echogenicity was recorded as positive.

Hepatomegaly was defined as a liver longitudinal span exceeding the 95th percentile for age and sex, measured in the midclavicular line during quiet respiration. Splenomegaly was

defined as a splenic longitudinal length exceeding the 95th percentile for age and sex, measured from the superior to the inferior pole in a sagittal plane while the child lay on the right side. Gallbladder abnormality was defined as non-visualization of the gallbladder on repeated examinations, a gallbladder length < 1.9 cm, or an irregular or lobulated gallbladder wall. The “triangular cord” sign-defined as an echogenic fibrous cord at the porta hepatis-was recorded separately as a distinct finding when present; it was not used as a criterion for gallbladder abnormality. Ascites was defined as anechoic fluid collections in the peritoneal cavity, most commonly in the perihepatic space (including Morison’s pouch, the hepatorenal recess between the liver and right kidney), perisplenic space, or pelvic cavity (the pouch of Douglas in females or the rectovesical pouch in males). The minimum detectable volume was approximately 5-10 mL.

The same two pediatric radiologists who performed the elastography analyzed the conventional ultrasound images independently. When opinions differed, an agreement was established after reviewing the images and clinical data.

Although the definitions of the triangular cord sign, gallbladder abnormality, and ascites were clarified, no formal re-review was performed because the original interpretations were already consistent with these definitions. To confirm this, the two radiologists who performed the original interpretations independently reviewed a random subset of 40 cases (10 from each group) using the clarified definitions. No discrepancies in classification were identified between the original and clarified criteria. Therefore, no cases required reclassification.

Statistical analysis

Continuous variables were expressed as mean $\pm$ SD after confirming normal distribution using the Shapiro-Wilk test ($P > 0.05$). Between-group comparisons of continuous variables were performed using the independent samples t-test, while categorical variables, expressed as numbers, proportions were assessed by chi-square or Fisher’s exact tests. All tests were two-sided, and $P < 0.05$ was considered significant.

Table 1. Demographic and baseline clinical characteristics of the NAFLD cohort

Finding	NAFLD Group (n = 129)	Healthy Group (n = 129)	t/ χ^2	P
Age (years)	12.53 $\pm$ 2.36	12.41 $\pm$ 2.45	0.421	0.674
Male sex [n (%)]	71 (55.04%)	73 (56.59%)	0.063	0.802
Body mass index (kg/m ²)	26.58 $\pm$ 3.85	18.94 $\pm$ 2.12	19.751	< 0.001
Waist circumference (cm)	82.36 $\pm$ 12.28	65.31 $\pm$ 8.62	12.906	< 0.001
Systolic blood pressure (mmHg)	111.28 $\pm$ 11.16	108.56 $\pm$ 10.74	1.994	0.047

BMI: body mass index; NAFLD: nonalcoholic fatty liver disease.

The diagnostic performance of LSM was evaluated using ROC analysis. Areas under the curve (AUC) were calculated for each cohort separately. The AUC, optimal cutoff value, sensitivity, specificity, and diagnostic accuracy were calculated for each cohort separately. The optimal LSM threshold was selected via the Youden index (sensitivity + specificity - 1), which optimizes their combined sum. For NAFLD cohort, the ROC analysis assessed the ability of LSM to distinguish children with NAFLD (positive group) from healthy children (negative group). For the BA cohort, the ROC analysis assessed the ability of LSM to distinguish infants with BA (positive group) from those with non-BA cholestasis (negative group). Statistical computations were carried out in SPSS (v29.0, IBM, Armonk, NY) and MedCalc (v20.0, Ostend, Belgium). 95% CIs for AUC values were calculated using the DeLong method. Significance was set at two-tailed $P < 0.05$.

For both ROC analyses, all calculations were based on unweighted complete-case data without imputation for missing values. The cutoff rule was defined as $LSM \geq$ the optimal threshold for a positive test result. Sensitivity, specificity, and accuracy were calculated from the raw 2×2 contingency tables. The 95% confidence intervals for sensitivity, specificity, and accuracy were computed using the Wilson score method, and those for likelihood ratios were calculated using the Koopman asymptotic score method. All diagnostic performance metrics were re-verified by two independent statisticians using the original individual-level data.

Results

NAFLD cohort

Table 1 summarizes the demographic and baseline clinical characteristics of NAFLD cohort. No significant differences were observed

between the NAFLD group and the healthy group in age, or sex (all $P > 0.05$). The NAFLD group had significantly higher BMI (26.58 $\pm$ 3.85 vs. 18.94 $\pm$ 2.12, $P < 0.001$) and waist circumference (82.36 $\pm$ 12.28 vs. 65.31 $\pm$ 8.62, $P < 0.001$) compared with healthy group. Systolic blood pressure was significantly higher in the NAFLD group (111.28 $\pm$ 11.16 vs. 108.56 $\pm$ 10.74 mmHg, $P = 0.047$).

Figure 3 shows the liver stiffness measurements and liver function tests in the NAFLD cohort. The NAFLD group showed significantly higher LSM (4.42 $\pm$ 0.92 vs. 3.74 $\pm$ 0.28, $P < 0.001$), ALT (64.18 $\pm$ 29.05 vs. 21.58 $\pm$ 7.86, $P < 0.001$), AST (52.82 $\pm$ 22.08 vs. 24.42 $\pm$ 6.54, $P < 0.001$), GGT (27.08 $\pm$ 13.16 vs. 19.46 $\pm$ 7.58, $P < 0.001$), and ALP (172.28 $\pm$ 47.16 vs. 156.96 $\pm$ 38.52, $P = 0.005$) compared to the healthy group. No significant differences were observed in total bilirubin or direct bilirubin (both $P > 0.05$).

Table 2 shows the metabolic findings and inflammatory markers in the NAFLD cohort. The NAFLD group had significantly higher total cholesterol (5.21 $\pm$ 0.95 vs. 3.99 $\pm$ 0.63, $P < 0.001$), triglycerides (1.87 $\pm$ 0.58 vs. 0.93 $\pm$ 0.29, $P < 0.001$), LDL-C (3.24 $\pm$ 0.77 vs. 2.35 $\pm$ 0.57, $P < 0.001$), CRP (3.2 $\pm$ 1.66 vs. 1.94 $\pm$ 0.86, $P < 0.001$), and ESR (14.62 $\pm$ 6.38 vs. 12.88 $\pm$ 5.34, $P = 0.018$), while HDL-C was significantly lower in the NAFLD group (0.97 $\pm$ 0.23 vs. 1.47 $\pm$ 0.29, $P < 0.001$). Fasting blood glucose was also slightly but significantly higher in NAFLD group (4.95 $\pm$ 0.71 vs. 4.79 $\pm$ 0.53, $P = 0.038$).

Table 3 summarizes the ultrasound morphologic findings in the NAFLD cohort. The prevalence of increased hepatic echogenicity (steatosis) was significantly higher in NAFLD group than in healthy group (88.37% vs. 0.78%, $P < 0.001$). Similarly, hepatomegaly (28.68% vs.

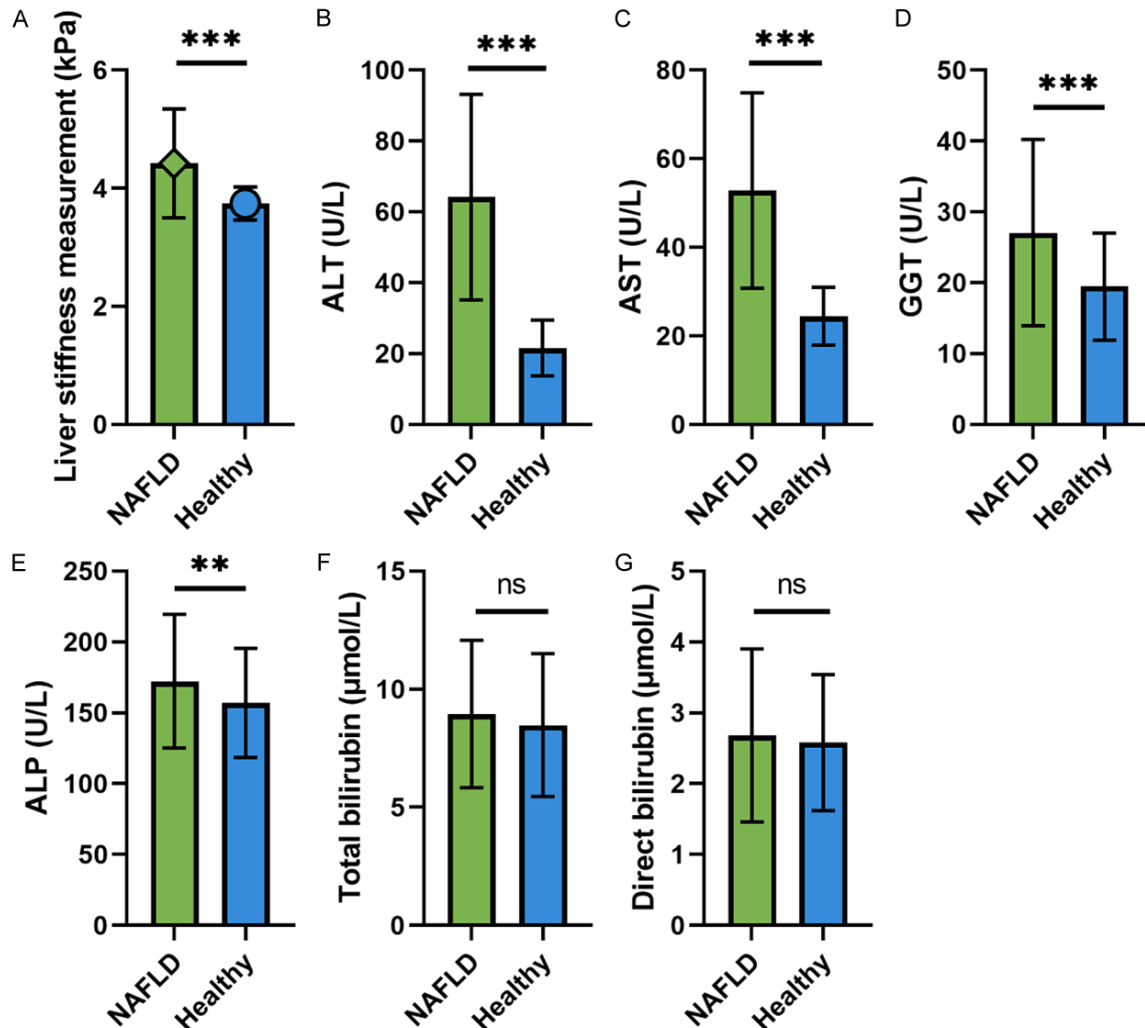


Figure 3. Liver stiffness measurement and liver function tests in the NAFLD cohort. A. Liver stiffness measurement; B. ALT; C. AST; D. GGT; E. ALP; F. Total bilirubin; G. Direct bilirubin. ns: no significant difference; **: $P < 0.01$; ***: $P < 0.001$. ALT: alanine aminotransferase; AST: aspartate aminotransferase; GGT: gamma-glutamyl transferase; ALP: alkaline phosphatase; NAFLD: nonalcoholic fatty liver disease; LSM: liver stiffness measurement; kPa: kilopascals.

7.75%, $P < 0.001$) and splenomegaly (10.08% vs. 2.33%, $P = 0.010$) were both significantly more common in NAFLD group.

Among the 129 children classified as having NAFLD, 15 (11.6%) did not show increased hepatic echogenicity on conventional ultrasound. These 15 children all had cardiometabolic risk factors (BMI $\geq$ 85th percentile, dyslipidemia, or impaired fasting glucose) and elevated ALT (≥ 40 U/L) on at least two separate occasions, with alternative causes of liver disease excluded; they therefore met the predefined composite diagnostic criteria for NAFLD despite having sonographically unde-

tectable steatosis. Conversely, one healthy control (0.8%) exhibited increased hepatic echogenicity on ultrasound. This participant was retained in the control group because liver function tests were normal (ALT 28 U/L, AST 26 U/L, total bilirubin 10.2 $\mu\text{mol/L}$) and no cardiometabolic risk factors were present; the isolated ultrasound finding was interpreted as a false-positive for steatosis, consistent with the known limitations of gray-scale ultrasound in pediatric populations. We have retained both the original ultrasound data and the original diagnostic classifications, as the diagnosis was established using the predefined composite clinical criteria rather than ultrasound alone.

Ultrasound elastography in pediatric liver disease

Table 2. Metabolic parameters and inflammatory markers in the NAFLD cohort

Finding	NAFLD Group (n = 129)	Healthy Group (n = 129)	t	P
Total cholesterol (mmol/L)	5.21 ± 0.95	3.99 ± 0.63	12.195	< 0.001
Triglycerides (mmol/L)	1.87 ± 0.58	0.93 ± 0.29	16.462	< 0.001
HDL-C (mmol/L)	0.97 ± 0.23	1.47 ± 0.29	15.351	< 0.001
LDL-C (mmol/L)	3.24 ± 0.77	2.35 ± 0.57	10.555	< 0.001
Fasting blood glucose (mmol/L)	4.95 ± 0.71	4.79 ± 0.53	2.083	0.038
CRP (mg/L)	3.2 ± 1.66	1.94 ± 0.86	7.654	< 0.001
ESR (mm/h)	14.62 ± 6.38	12.88 ± 5.34	2.373	0.018

HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; CRP: C-reactive protein; ESR: erythrocyte sedimentation rate; NAFLD: nonalcoholic fatty liver disease.

Table 3. Ultrasound morphologic findings in the NAFLD cohort

Finding	NAFLD Group (n = 129)	Healthy Group (n = 129)	χ^2	P
Increased hepatic echogenicity (steatosis), n (%)	114 (88.37%)	1 (0.78%)	200.328	< 0.001
Hepatomegaly, n (%)	37 (28.68%)	10 (7.75%)	18.966	< 0.001
Splenomegaly, n (%)	13 (10.08%)	3 (2.33%)	6.663	0.010

NAFLD: nonalcoholic fatty liver disease.

Table 4. Demographic and baseline clinical characteristics of the BA cohort

Finding	BA Group (n = 83)	Non-BA Cholestasis Group (n = 83)	t/ χ^2	P
Age (months)	2.86 ± 1.41	2.65 ± 1.35	0.967	0.335
Male sex [n (%)]	45 (54.22%)	43 (51.81%)	0.097	0.756
Body weight (kg)	4.52 ± 0.83	4.37 ± 0.78	1.200	0.232
Body length (cm)	54.28 ± 3.52	53.86 ± 3.41	0.771	0.442
Age at onset of jaundice (days)	12.35 ± 5.28	10.86 ± 4.92	1.887	0.061

BA: biliary atresia.

Table 5. Liver stiffness measurement and liver function tests in the BA cohort

Finding	BA Group (n = 83)	Non-BA Cholestasis Group (n = 83)	t	P
Liver stiffness measurement (kPa)	13.58 ± 8.46	6.31 ± 1.02	7.771	< 0.001
ALT (U/L)	118.52 ± 54.36	68.46 ± 28.62	7.425	< 0.001
AST (U/L)	141.38 ± 70.42	85.72 ± 32.54	6.536	< 0.001
GGT (U/L)	291.52 ± 115.58	102.68 ± 48.84	13.711	< 0.001
ALP (U/L)	458.86 ± 162.48	218.58 ± 78.63	12.127	< 0.001
Total bilirubin (μmol/L)	141.42 ± 60.48	86.56 ± 35.38	7.134	< 0.001
Direct bilirubin (μmol/L)	102.68 ± 44.36	58.46 ± 24.68	7.936	< 0.001

BA: biliary atresia; ALT: alanine aminotransferase; AST: aspartate aminotransferase; GGT: gamma-glutamyl transferase; ALP: alkaline phosphatase; LSM: liver stiffness measurement; kPa: kilopascals.

BA cohort

Table 4 presents demographic and baseline clinical characteristics of the BA cohort. No significant differences were observed between BA group and non-BA cholestasis group in age,

sex, body weight, body length, or age at onset of jaundice (all $P > 0.05$).

Table 5 shows the liver stiffness measurement and liver function tests in the BA cohort. The BA group had significantly higher LSM (13.58 ±

Table 6. Metabolic findings and inflammatory markers in the BA cohort

Finding	BA Group (n = 83)	Non-BA Cholestasis Group (n = 83)	t	P
Total cholesterol (mmol/L)	3.49 ± 0.73	3.97 ± 0.83	3.937	< 0.001
Triglycerides (mmol/L)	0.99 ± 0.35	1.23 ± 0.41	4.023	< 0.001
HDL-C (mmol/L)	1.43 ± 0.34	1.33 ± 0.37	1.788	0.076
LDL-C (mmol/L)	1.91 ± 0.57	2.17 ± 0.61	2.799	0.006
Fasting blood glucose (mmol/L)	4.29 ± 0.49	4.51 ± 0.51	2.751	0.007
CRP (mg/L)	12.62 ± 5.42	6.88 ± 3.02	8.438	< 0.001
ESR (mm/h)	32.08 ± 12.16	22.52 ± 9.58	5.624	< 0.001

BA: biliary atresia; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; CRP: C-reactive protein; ESR: erythrocyte sedimentation rate.

Table 7. Ultrasound morphologic findings in the BA cohort

Finding	BA Group (n = 83)	Non-BA Cholestasis Group (n = 83)	χ^2	P
Hepatomegaly, n (%)	66 (79.52%)	54 (65.06%)	4.330	0.037
Gallbladder abnormality, n (%)	71 (85.54%)	15 (18.07%)	75.665	< 0.001
Splenomegaly, n (%)	45 (54.22%)	23 (27.71%)	12.056	< 0.001
Ascites, n (%)	20 (24.1%)	10 (12.05%)	4.069	0.044

BA: biliary atresia.

Table 8. ROC analysis of LSM for distinguishing NAFLD from healthy children

Finding	Value	95% CI
AUC	0.865	0.820-0.910
Optimal cutoff value (kPa)	3.95	
Rule for positive test	LSM ≥ cutoff	
Sensitivity (%)	68.2	60.1-76.0
Specificity (%)	85.2	78.1-90.4
Diagnostic accuracy (%)	76.8	71.2-81.6
Positive likelihood ratio	4.64	3.23-6.66
Negative likelihood ratio	0.37	0.29-0.48

LSM: liver stiffness measurement; NAFLD: nonalcoholic fatty liver disease; CI: confidence interval; kPa: kilopascals; AUC: Area under the curve.

8.46 vs. 6.31 ± 1.02 , $P < 0.001$), ALT (118.52 ± 54.36 vs. 68.46 ± 28.62 , $P < 0.001$), AST (141.38 ± 70.42 vs. 85.72 ± 32.54 , $P < 0.001$), GGT (291.52 ± 115.58 vs. 102.68 ± 48.84 , $P < 0.001$), ALP (458.86 ± 162.48 vs. 218.58 ± 78.63 , $P < 0.001$), total bilirubin (141.42 ± 60.48 vs. 86.56 ± 35.38 , $P < 0.001$), and direct bilirubin (102.68 ± 44.36 vs. 58.46 ± 24.68 , $P < 0.001$) compared to the non-BA cholestasis group.

Table 6 summarizes the metabolic findings and inflammatory markers in BA cohort. BA group had significantly lower total cholesterol (3.49 ± 0.73 vs. 3.97 ± 0.83 , $P < 0.001$), triglycerides (0.99 ± 0.35 vs. 1.23 ± 0.41 , $P < 0.001$),

LDL-C (1.91 ± 0.57 vs. 2.17 ± 0.61 , $P = 0.006$), and fasting blood glucose (4.29 ± 0.49 vs. 4.51 ± 0.51 , $P = 0.007$), while CRP (12.62 ± 5.42 vs. 6.88 ± 3.02 , $P < 0.001$) and ESR (32.08 ± 12.16 vs. 22.52 ± 9.58 , $P < 0.001$) were significantly higher in the BA group. No significant difference was observed in HDL-C ($P = 0.076$).

Table 7 presents ultrasound morphologic findings in the BA cohort. The BA group had a significantly higher prevalence of hepatomegaly (79.52% vs. 65.06%, $P = 0.037$), gallbladder abnormality (85.54% vs. 18.07%, $P < 0.001$), splenomegaly (54.22% vs. 27.71%, $P < 0.001$), and ascites (24.1% vs. 12.05%, $P = 0.044$) compared to the non-BA cholestasis group.

Diagnostic performance of ultrasound elastography

Table 8 shows the ROC analysis of LSM for distinguishing NAFLD from healthy children. The area under the curve was 0.865 (95% CI: 0.820-0.910). At the optimal cutoff value of 3.95 kPa (using the rule LSM ≥ cutoff as positive), the sensitivity was 68.2% (95% CI: 60.1-76.0%), specificity was 85.2% (95% CI: 78.1-90.4%), and diagnostic accuracy was 76.8%

Table 9. 2 × 2 diagnostic table for LSM in distinguishing NAFLD from healthy children at a cutoff of 3.95 kPa (Rule: LSM ≥ 3.95 kPa as Positive)

Finding	NAFLD Group (n = 129)	Healthy Group (n = 129)	Total
LSM ≥ 3.95 kPa (Positive)	88	19	107
LSM < 3.95 kPa (Negative)	41	110	151
Total	129	129	258

LSM: liver stiffness measurement; NAFLD: nonalcoholic fatty liver disease.

Table 10. ROC analysis of LSM for distinguishing BA from non-BA cholestasis

Finding	Value	95% CI
AUC	0.890	0.865-0.935
Optimal cutoff value (kPa)	12.0	
Rule for positive test	LSM ≥ cutoff	
Sensitivity (%)	84.3	74.7-91.0
Specificity (%)	88.0	79.0-93.6
Diagnostic accuracy (%)	86.1	80.0-90.6
Positive likelihood ratio	7.00	4.11-11.93
Negative likelihood ratio	0.18	0.11-0.30

BA: biliary atresia; LSM: liver stiffness measurement; CI: confidence interval; kPa: kilopascals; AUC: Area under the curve.

Table 11. 2 × 2 diagnostic table for LSM in distinguishing BA from non-BA cholestasis at a cutoff of 12.0 kPa (Rule: LSM ≥ 12.0 kPa as Positive)

Finding	BA Group (n = 83)	Non-BA Cholestasis Group (n = 83)	Total
LSM ≥ 12.0 kPa (Positive)	70	10	80
LSM < 12.0 kPa (Negative)	13	73	86
Total	83	83	166

BA: biliary atresia; LSM: liver stiffness measurement.

(95% CI: 71.2-81.6%). The positive likelihood ratio was 4.64 (95% CI: 3.23-6.66) and the negative likelihood ratio was 0.37 (95% CI: 0.29-0.48). The corresponding 2 × 2 table at this cutoff is shown in **Table 9**.

Table 10 presents the ROC analysis of LSM for distinguishing BA from non-BA cholestasis. The area under the curve was 0.890 (95% CI: 0.865-0.935). Using the optimal cutoff value of 12.0 kPa (rule: LSM ≥ cutoff as positive), the sensitivity was 84.3% (95% CI: 74.7-91.0%), specificity was 88.0% (95% CI: 79.0-93.6%), and diagnostic accuracy was 86.1% (95% CI: 80.0-90.6%). The positive likelihood ratio was 7.00 (95% CI: 4.11-11.93) and the negative

likelihood ratio was 0.18 (95% CI: 0.11-0.30). The corresponding 2 × 2 diagnostic table at this cutoff is shown in **Table 11**.

Correlation analysis

Pearson correlation analysis was performed to explore the relationships between LSM and key clinical findings. In the NAFLD cohort (n = 258), LSM was positively correlated with BMI (r = 0.42, 95% CI: 0.31-0.52, P < 0.001), ALT (r = 0.38, 95% CI: 0.27-0.48, P < 0.001), and AST (r = 0.35, 95% CI: 0.24-0.45, P < 0.001), and negatively correlated with HDL-C (r = -0.33, 95% CI: -0.44 to -0.22, P < 0.001). In the BA cohort (n = 166), LSM showed significant positive correlations with GGT (r = 0.56, 95% CI: 0.44-0.66, P < 0.001) and total bilirubin (r = 0.48, 95% CI: 0.35-0.59, P < 0.001). No significant correlation was found with age or sex in either cohort. These correlation analyses were exploratory; no adjustment for multiple comparisons was applied.

Discussion

The present study aimed to evaluate the clinical significance of ultrasound elastography in assessing liver stiffness in pediatric patients with NAFLD and BA. The

findings highlight important differences in LSM and other clinical findings between these two conditions and their respective control groups.

NAFLD cohort

Children with NAFLD exhibited higher BMI and waist circumference compared to healthy controls. This suggests that obesity is a critical factor in the development of liver stiffness. Obesity promotes hepatic fat deposition, which may progress to steatosis, inflammation, and fibrosis [13-15]. The observed elevations in ALT, AST, GGT, and ALP indicate ongoing liver injury and inflammation, suggesting that these processes contribute to liver stiffness. Our results

corroborate the established pathophysiology of NAFLD, in which metabolic factors are pivotal in hepatic damage [16-18]. The presence of increased hepatic echogenicity, hepatomegaly, and splenomegaly further supports the notion that NAFLD is associated with significant structural liver changes. These structural changes may result from inflammation and fibrosis induced by intrahepatic lipid deposition [19, 20].

The higher levels of total cholesterol, triglycerides, LDL-C, CRP, and ESR observed in the NAFLD group reflect systemic metabolic dysregulation and inflammation. These metabolic factors likely exacerbate liver damage by promoting oxidative stress and triggering hepatic inflammation. Oxidative stress may trigger stellate cell activation, driving extracellular matrix (ECM) production and contributing to fibrosis [21, 22]. Inflammation driven by adipokines, cytokines, and chemokines further damages hepatocytes and promotes fibrogenesis [23]. The presence of hepatomegaly and splenomegaly indicates portal hypertension, which is a common complication of advanced liver disease [24, 25].

BA cohort

The BA cohort demonstrated markedly higher LSM, ALT, AST, GGT, ALP, total bilirubin, and direct bilirubin levels compared to infants with non-BA cholestasis. These differences likely reflect the distinct pathophysiology of BA, characterized by progressive bile duct destruction and subsequent liver fibrosis. The severe elevation of liver enzymes and bilirubin indicates extensive liver damage and cholestasis, which contribute to increased liver stiffness. Unlike NAFLD, which is driven by metabolic factors, BA involves an immune-mediated process leading to bile duct obliteration and subsequent liver fibrosis. Bile stasis resulting from bile duct obstruction induces hepatocyte apoptosis and necrosis, leading to liver injury. The ensuing liver injury triggers a reparative response characterized by fibrosis and cirrhosis [26, 27].

The lower levels of total cholesterol, triglycerides, LDL-C, and fasting blood glucose in the BA group may reflect impaired bile acid secretion and malabsorption, which are common features of advanced cholestatic liver disease. Elevated CRP and ESR levels suggest systemic

inflammation, which could exacerbate liver damage and fibrosis. The higher prevalence of hepatomegaly, gallbladder abnormalities, splenomegaly, and ascites in the BA group highlights the extensive liver involvement and portal hypertension seen in this condition. These findings emphasize the need for early intervention and surgical correction in BA to prevent irreversible liver damage [28, 29].

Comparison and clinical implications

When comparing the two cohorts, LSM elevation was modest in NAFLD and associated with metabolic factors, whereas in BA it was markedly higher and correlated with cholestatic liver injury, reflecting distinct pathogenic pathways. These differences underscore the need for disease-specific interpretation of LSM values in pediatric practice.

Ultrasound elastography proved valuable for distinguishing NAFLD from healthy controls and BA from non-BA cholestasis. The ability to measure liver stiffness non-invasively provides important insight into disease severity and can guide clinical decision-making. In NAFLD, increased liver stiffness reflects the degree of fibrosis and inflammation, which are critical determinants of disease progression. Early detection of liver stiffness using elastography may enable timely interventions such as lifestyle modifications and pharmacologic treatments to mitigate liver damage [30, 31]. For BA, the high diagnostic accuracy of elastography suggests its potential use in identifying patients with BA among those with neonatal cholestasis. Whether LSM can guide decisions regarding urgent surgical intervention, monitor treatment response, or improve long-term outcomes remains to be established in future prospective studies [32, 33].

Despite the promising findings, several limitations of this study should be considered. Retrospective studies carry intrinsic limitations (selection and confounding) that may limit the applicability of results. The use of healthy controls, rather than children with other hepatic or metabolic conditions, may have introduced spectrum bias and could overestimate the diagnostic performance of LSM for NAFLD. Therefore, our conclusions regarding NAFLD are appropriately limited to discrimination between established NAFLD cases and healthy

children. Although our results demonstrate the discriminatory performance of LSM, this study was not designed to establish its independent or incremental clinical value beyond standard clinical assessment. Multivariable adjustment was not performed because of the case-control design and the limited number of events in each cohort. Claims regarding clinical utility should therefore be interpreted with caution. Prospective studies with larger cohorts are needed to corroborate these findings and examine the long-term consequences of liver stiffness on clinical outcomes. While ultrasound elastography offers a non-invasive method for assessing liver stiffness, it does not provide detailed information on the histopathological features of liver disease. Combining elastography with other imaging modalities or liver biopsy may enhance diagnostic accuracy and provide a more comprehensive assessment of liver health. It should be noted that one operator performed all examinations for the NAFLD cohort (children) and the other performed all examinations for the BA cohort (infants). Thus, operator is confounded with cohort type (children vs. infant). This does not invalidate the within-cohort diagnostic comparisons, but it limits direct interpretation of absolute LSM differences between the NAFLD and BA cohorts. This limitation is acknowledged. This study lacked histopathologic confirmation (liver biopsy) and fibrosis staging, as well as long-term follow-up data, which would have provided additional validation of LSM findings. Future prospective studies incorporating these endpoints are warranted. The study focused primarily on immediate endpoints; the durability of stiffness improvements remains to be explored. Long-term follow-up data would provide valuable insight into the durability of these benefits and the potential for delayed complications.

This study suggests that ultrasound elastography is a useful tool for evaluating liver stiffness in pediatric patients with NAFLD and BA. The differences in liver stiffness between these conditions reflect distinct pathophysiologic processes and highlight the importance of early diagnosis and tailored management strategies. Future studies should aim to identify additional biomarkers and imaging techniques that complement elastography, providing a more holistic view of liver health and guiding optimal

treatment decisions. Through systematic evaluation and adaptation of current practices, we can strive toward better health outcomes for pediatric patients with liver diseases.

Conclusion

Ultrasound elastography demonstrated good diagnostic accuracy for discriminating NAFLD from healthy children and BA from non-BA cholestasis. In NAFLD, obesity and metabolic abnormalities were associated with increased LSM, while in BA, LSM elevation correlated with markers of cholestatic liver injury. These findings support a role of 2D-SWE as a non-invasive tool for discriminating these two distinct pediatric liver diseases. However, further prospective studies with histologic validation and longitudinal follow-up are needed to confirm these findings and explore the broader clinical implications of LSM measurements in pediatric liver diseases.

Disclosure of conflict of interest

None.

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