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Validation of Transient Elastography Cut-points to Assess Advanced Liver Fibrosis in Children and Young Adults: The Boston Children's Hospital Experience

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Abstract

OBJECTIVE—To derive an optimal LSM cut-point to discriminate METAVIR F4 and to validate both cut-points in a separate cohort.

STUDY DESIGN—Patients at Boston Children's Hospital with LSM from 2006–2016 and liver biopsy 12 months prior to screening were eligible. Patients enrolled 2006–2011 were used to calibrate LSM cut-points, and those enrolled 2011–2016 for validation. Diagnostic performance was assessed by receiver operating curve analysis.

RESULTS—267 subjects were enrolled (97 calibration, 170 validation). The cohorts were similar with 54% male, ages 0–29 years (median 13) and liver diseases including 21% autoimmune, 19% viral, 11% non-alcoholic fatty liver, 9% cholestatic, and 9% PSC. Cut-points to discriminate F3–F4 and F4 were >8.6 kPa and >11.5 kPa with 81% and 84% accuracy, respectively. Applied to the validation cohort, accuracy was 67% and 75%, respectively. In 44 fasted subjects, the accuracy was 73% and 80% respectively.

CONCLUSION—This study validates previously determined LSM cut-points of 8.6 kPa and 11.5 kPa to predict METAVIR F3–F4 and F4 fibrosis in children and young adults in separate cohorts. With increasing data on the utility and validity of LSM in children, TE may help identify patients with higher risk of advanced fibrosis and those who need liver biopsy assessment and/or surveillance for the complications of cirrhosis in a variety of liver disorders.

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Liver fibrosis is associated with complications, morbidity, and mortality in chronic liver disease. Histopathologic assessment of fibrosis on liver biopsy remains the reference standard for determining the severity of fibrosis, yet is associated with complications and sampling error.^{2–5} Moreover, children are exposed to additional risk with liver biopsy due to need for anesthesia or sedation and possibly post-procedure hospitalizations. Transient elastography is an ultrasound-based tool that has been shown to rapidly and reproducibly measure liver stiffness, which reflects hepatic fibrosis^{6–7}. Multiple studies have shown that liver stiffness measurement (LSM) by TE can predict hepatic fibrosis in children with various chronic liver diseases^{1,8–10}. Each study has reported slightly different LSM cut-points to identify advanced fibrosis. These studies involved smaller sample sizes, use of limited patient populations and mainly used the adult medium (M) probe only. We reported an optimal LSM cut-point >8.6 kPa to detect METAVIR F3–F4 with a high area under the receiver operator characteristic curve (AUROC=0.84)¹. Although cut-points have been validated in adult populations, these cut-points have not been validated in pediatric populations with liver disease¹¹.

The aim of the present study was to validate our previously reported liver stiffness measurement cut-point to detect advanced liver fibrosis (F3–F4) in children and young adults. In addition, we aimed to identify and validate an additional LSM cut-point to detect cirrhosis (F4) in children and young adults. Although there are many published reports using TE in children, most do not include the reference standard assessment for comparison.

Methods

This was a prospective 2-phase study of unselected children and young adults at Boston Children's Hospital (BCH) who underwent LSM over a 10 year period from 2006 to 2016. All patients were required to have had a liver biopsy within 12 months of enrollment and been available to a study investigator for recruitment. Patients who had an uninterpretable or unavailable biopsy specimen, who lacked critical clinical and/or biochemical data, or who were not candidates for TE because of a technically invalid measurement, ascites, morbid obesity (body mass index > 40 kg/m²), pregnancy or implantable cardiac device were excluded according to manufacturer guidelines. Patients who had undergone Fontan surgery were also excluded given the known high degree of hepatic stiffness in these patients^{12–13}. The calibration cohort included patients enrolled from August 2006–April 2011 who were used to develop LSM cut-points for discriminating F3–F4 and F4 fibrosis. Patients enrolled from April 2011–March 2016 comprised the validation group and were used to test the diagnostic accuracy of these cut-points. This study was approved by the Boston Children's Hospital Institutional Review Board. Written informed consent was obtained from parents, legal guardians, or patients 18 years of age. Patient assent was obtained when appropriate.

Liver histology

All patients underwent liver biopsy for clinical indications. The METAVIR system was used to stage fibrosis by 5 hepatopathologists blinded to LSM. Scoring to stage liver fibrosis was as follows: F0, no fibrosis; F1, portal fibrosis without septa; F2, portal fibrosis and few

septa; F3, numerous septa without cirrhosis; F4, cirrhosis. Scores of F3 or F4 were considered advanced fibrosis.

Liver stiffness measurements

Liver stiffness was measured by TE (FibroScan, Echosens, Paris, France), an ultrasound-based technique which involves placing an ultrasonic transducer in a right intercostal space to transmit a vibration of mild amplitude and low frequency. The vibration on the skin surface creates a shear wave which propagates through the right lobe of the liver. The velocity of propagation is directly related to tissue stiffness; the harder the tissue (as in hepatic fibrosis) the faster the shear wave propagates. Eight to ten valid LSMs were obtained and reported as a median value in kiloPascals (kPa). Adequacy of measurement was assessed by the TE device. LSM was performed by 6 trained study investigators who were certified by the manufacturer and blinded to the liver biopsy results. TE probe size selection was based on thoracic perimeter (TP); the M (medium) probe was used if TP > 75 cm and the S (small) if TP ≤ 75 cm. Prior to November 2009, when the S probe became available, no patients weighing < 50 pounds were included.

Statistical Analyses

Patient characteristics and primary diagnostic indications for liver biopsy are presented as N (%) if categorical, and either mean ± standard deviation (SD) or median with interquartile range (IQR) if continuous. Comparison for these characteristics between the calibration and validation cohorts was made by Pearson chi-square test or Student t-test, or by their nonparametric analogs, Fisher exact test and Wilcoxon rank-sum test.

Box-whisker plots were used to illustrate the distribution of LSM across the 5 METAVIR stages, and comparisons between the calibration and validation cohorts was made by Student t-test. Differences in LSM within each METAVIR stage were investigated with two-way analysis of variance (ANOVA) using an interaction term for cohort and METAVIR. Although LSM was right-skewed, results from nonparametric analysis and analysis of LSM after transforming to normal scores were consistent with the parametric results, and only the latter are reported¹⁴.

The calibration cohort was used to construct receiver operating characteristic (ROC) curves to evaluate the ability of TE to discriminate METAVIR stages F3–F4 from stages F0–F2 and F4 from stages F0–F3. The area under the ROC curve (AUROC) is reported with a 95% confidence interval (CI) to compare the curve with the diagonal (AUROC=0.5, indicating predictive ability no better than a coin flip). An optimal cut-point was determined by minimizing the probability of a false positive TE result and maximizing the probability of a true positive TE result. These cut-points were then used to construct 2×2 contingency tables comparing the disease status (presence/absence based on METAVIR dichotomy) to the TE result (positive/negative based on cut-point dichotomy). Diagnostic performance of TE was assessed by sensitivity (Sn), specificity (Sp), accuracy (Ac), positive predictive value (PPV), negative predictive value (NPV), positive likelihood ratio (LR+), and negative likelihood ratio (LR-), and shown with 95% confidence interval (CI)^{15–16}. Validation was assessed for

the entire validation cohort as well as for a subgroup of subjects who were fasted prior to TE. Diagnostic performance characteristics were compared by the Fisher exact test.

All data analysis was performed and figures prepared with SAS version 9.4 (SAS Institute, Cary, North Carolina). All tests of significance were 2-sided, with $P < .05$ indicating statistical significance.

Results

A total of 321 subjects were screened from 2006 – 2016, of whom 54(17%) were excluded due to inability to obtain a valid liver biopsy or LSM, resulting in a sample of 267 subjects available for analysis (Figure 1; available at www.jpeds.com). The calibration cohort used to determine optimal cut-points for discriminating F3–F4 and F4 fibrosis consisted of 97 subjects¹; the cut-points were validated using a cohort of 170 subjects.

Demographic characteristics of the study population and primary diagnostic indications for liver biopsy are shown in Table I. 230 subjects (86%) were < 18 years of age. Subjects in the validation cohort were less likely to be Caucasian race (59% vs 72%; $P=0.03$), and to have had a liver transplant (1% vs 5%; $P=0.03$) than those in the calibration cohort. The distribution of characteristics was otherwise statistically similar across the two cohorts. All METAVIR stages were well represented. Approximately one-third of subjects had advanced fibrosis (stage F3–F4), 35% in the calibration cohort and 28% in the validation cohort ($P=0.25$).

Figure 2 shows the distribution of LSM scores by METAVIR stage F0–F4 in both the calibration and validation cohorts. Although the protocol specified that LSM was to be obtained within 1 year of biopsy, the median interval between the time of biopsy and LSM was only 46 days (IQR 19 – 130) with 81% of subjects having the LSM performed within 6 months of biopsy. LSM was statistically similar in the 2 cohorts. The calibration cohort had an overall mean LSM of 12.3 ± 1.4 compared with the validation cohort of 12.8 ± 1.1 ($P=0.80$ by t-test). There was no difference between cohorts when examined within METAVIR stages ($P=0.78$ by ANOVA 2-way interaction). However, although LSM was statistically similar among METAVIR stages F0–F2 for both cohorts combined ($P = 0.20$ for all pairwise comparisons among F0, F1 and F2), LSM for F3 was on average 4.0 kPa higher than the mean LSM for F0–F2 (13.7 ± 1.8 vs. 9.7 ± 0.9 kPa, $P=0.05$), and LSM for F4 was more than double the mean for F0–F2 (26.6 ± 2.0 vs. 9.7 ± 0.9 kPa, $P<0.0001$; comparisons not shown).

Cut-points were derived from receiver operating characteristic curves to predict F3–F4 (advanced fibrosis) and F4 (cirrhosis) using data from the calibration cohort. Optimal cut-points to predict advanced fibrosis and cirrhosis were determined to be LSM >8.6 kPa and >11.5 kPa, respectively, with areas under the ROC curve of 0.85 (95% CI 0.76 – 0.93) and 0.87 (95% CI 0.79 – 0.96) (Figure 3; available at www.jpeds.com). Diagnostic performance characteristics for these cut-points are shown in Table 2 for both the calibration and validation cohorts. The accuracy for predicting F3–F4 advanced fibrosis and F4 fibrosis in the calibration cohort was 81.4% (95% CI 72.3 – 88.6%) and 83.5% (95% CI 74.6 – 90.3%),

respectively, compared with 67.1% (95% CI 59.4 – 74.1%) and 75.3% (95% CI 68.1 – 81.6%) in the validation cohort.

Because the importance of at least 3 hours of fasting prior to LSM was demonstrated in adult studies while data collection for the validation cohort was already underway, the protocol was amended to include fasting status, and is available for 44 (26%) validation subjects. Analysis of variance was used to investigate differential effects in the calibration cohort or METAVIR status by fasting. Differences were found only for the main effect of METAVIR status; no differences were attributable to fasting ($P = 0.76$) (Table 3; available at www.jpeds.com).

With fasting, accuracy for the F3–F4 advanced fibrosis and F4 cirrhosis cut-points was 72.7% (95% CI 57.2 – 85.0%) and 79.5% (95% CI 74.7 – 90.2%) respectively (Table 4).

Discussion

Transient elastography is a tool used to measure liver stiffness and has been shown to rapidly and reliably assess fibrosis in adults and children. There have been several studies using TE to measure LSM in children with various liver diseases, with differing cut-points for advanced fibrosis. Some of the variability in published cut-points has likely been the result of studies of patients with specific liver diseases, unavailability of the S-probe for smaller children and the under-representation of advanced fibrosis.

This study evaluated a previously published LSM cut-point and validate its performance in an independent pediatric cohort. Overall, LSM diagnostic performance characteristics were better for the calibration cohort in comparison to the validation cohort. However, the differences in diagnostic performance between the calibration and validation cohorts were only statistically different for PPV (both cut-points), specificity (F3–F4 cut-point only) and accuracy (F3–F4 cut-point only). There were no statistical differences in diagnostic performance between the calibration and fasting cohort, reflecting both improved performance and potentially reduced power due to a reduction in sample size.

Our results were similar to that of a validation study of TE in adults. In 2015, a prospective 2-phase, multi-center study of transient elastography in adult patients with chronic hepatitis C or B included 188 subjects in the developmental phase and 560 subjects in the validation phase.¹¹ In phase 1, the cut-point to predict F2 fibrosis was 8.4 kPa with AUROC 0.89, 82% sensitivity, 79% specificity, and to predict F4 fibrosis was 12.8 kPa with AUROC 0.92, 84% sensitivity, 86% specificity. When these cut-points were tested in the validation cohort, the F2 fibrosis cut-point had 58% sensitivity, 75% specificity and for the F4 fibrosis cut-point 76% sensitivity and 85% specificity.

This study has several limitations. This study cohort includes a heterogeneous group of liver disease. The study performed in an array of diseases is representative of those seen in our clinical practice, but may over- or under-estimate accuracy in any particular disorder or type of disorder. Another study limitation is potential selection bias. Many patients who underwent liver biopsy were not available to recruitment which could have results in selection bias. In addition, 44 subjects had liver stiffness measurements that either could not

be obtained or were not valid, thus also introducing potential selection bias in the cohort. Though an additional confounding factor could be the possibility of changing status of liver disease in the interim between liver biopsy and LSM, the median time between liver biopsy and LSM was only 46 days (IQR 9–130 days) with 81% of subjects having LSM within 6 months of their biopsy. In addition, although liver biopsies were reviewed by 5 blinded hepatopathologists, they used a validated, standardized histologic scoring system thereby minimizing inter-observer variability.

Unfortunately, the importance of fasting was not established until the validation protocol was underway, and LSM under fasting conditions is not uniformly available for all subjects. As noted, the manufacturer now recommends fasting for at least 3 hours before LSM due to potential overestimation of LSM in the post-prandial state. Because these recommendations were not published until after the completion of the calibration study, this could have resulted in compromise of the accuracy of LSM and the derived cut-points. Similarly, recent studies in adults and children have shown that LSM can be further confounded by other components of liver disease. Some of these studies highlight the association between hepatic inflammation and the overestimate of LSM by TE^{17–19}.

This study validates LSM cut-points of 8.6 kPa and 11.5 kPa to predict advanced fibrosis and cirrhosis in separate cohorts of children and young adults with liver disease. TE has been known to be a safe, well-tolerated technique in both adults and children. With this new information, more formal guidelines can be developed to help use TE values within clinical practice to help identify those pediatric patients with advanced fibrosis or cirrhosis. When used in combination in clinical data, TE could be used to assess the risk of cirrhosis in children. With increasing data on the utility and validity of liver stiffness measurement in children, TE may help identify those patients with higher risk of advanced fibrosis and those who need liver biopsy assessment and/or surveillance for the complications of cirrhosis in a variety of liver disorders.

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Figure 1.
Derivation of study population

Figure 2.
Distribution of LSM by METAVIR stage for each cohort.

A. METAVIR F3-F4 (advanced fibrosis).
Area under the curve = 0.85

B. METAVIR F4 (cirrhosis).
Area under the curve = 0.87

Figure 3.

Receiver operating characteristic curves based on the calibration cohort. Optimal cut-points for predicting METAVIR F3–F4 (advanced fibrosis) and F4 (cirrhosis) are shown.

Table 1

Comparison of calibration and validation cohorts.

Characteristic	Unknown (calibration, validation)	Calibration (n=97)	Validation (n=170)	P
Female sex	0, 0	52 (54%)	72 (42%)	0.08
White	0, 13	70 (72%)	92 (59%)	0.03
Age at TE, mean (SD)	0, 0	12.5±6.5	11.8±6.6	0.38
0–2y		13 (13%)	31 (18%)	
3–6y		9 (9%)	13 (8%)	
7–11y		23 (24%)	24 (14%)	
12–17y		38 (39%)	79 (46%)	
18y		14 (14%)	23 (14%)	
Liver disease diagnosis	0, 0			
Autoimmune		20 (21%)	33 (20%)	0.85
Viral		19 (20%)	31 (18%)	0.79
Cholestasis		13 (13%)	16 (9%)	0.31
PSC		12 (12%)	17 (10%)	0.56
Fatty liver		8 (8%)	23 (14%)	0.20
Post-transplantation		5 (5%)	1 (1%)	0.03
BMIZ, mean±SD	9, 32	0.39±1.26	0.20±1.46	0.31
ALT at TE, IU/L, median (IQR)	2, 12	87 (46, 197)	74 (40, 150)	0.06
INR at TE, median (IQR)	6, 40	1.04 (0.99, 1.12)	1.04 (0.98, 1.11)	0.63
Platelets at TE, ×10 ⁹ /L, median (IQR)	7, 27	242 (191, 308)	259 (190, 341)	0.32
METAVIR	0, 0			0.18
F0		11 (11%)	36 (21%)	
F1		34 (35%)	52 (31%)	
F2		18 (19%)	34 (20%)	
F3		16 (16%)	29 (17%)	
F4		18 (19%)	19 (11%)	

Table 2

Performance of cut-points to predict F3–F4 (advanced fibrosis) and F4 (cirrhosis).

	LSM cut-point	Cohort	N	Sn (%)	Sp (%)	PPV (%)	NPV (%)	Ac (%)	LR+	LR–
F3–F4	>8.6 kPa	Calibration	38 (39%)	79.4 (62.1, 91.3)	82.5 (70.9, 90.9)	71.1 (58.3, 81.2)	88.1 (79.2, 93.6)	81.4 (72.3, 88.6)	4.55 (2.59, 7.99)	0.25 (0.13, 0.49)
		Validation	76 (45%)	70.8 (55.9, 83.0)	65.6 (56.4, 73.9)	44.7 (37.4, 52.3)	85.1 (78.3, 90.0)	67.1 (59.4, 74.1)	2.06 (1.52, 2.79)	0.44 (0.28, 0.70)
		<i>P</i> value		0.45	0.02	0.01	0.64	0.02		
F4	>11.5 kPa	Calibration	28 (29%)	83.3 (58.6, 96.4)	83.5 (73.5, 90.9)	53.6 (40.3, 66.4)	95.7 (88.6, 98.4)	83.5 (74.6, 90.3)	5.06 (2.96, 8.67)	0.20 (0.07, 0.56)
		Validation	53 (31%)	78.9 (54.4, 93.9)	74.8 (67.1, 81.5)	28.3 (21.6, 36.1)	96.6 (92.2, 98.5)	75.3 (68.1, 81.6)	3.14 (2.19, 4.50)	0.28 (0.12, 0.68)
		<i>P</i> value		1.00	0.18	0.03	0.71	0.13		

Performance characteristics include sensitivity (Sn), specificity (Sp), positive predictive value (PPV), negative predictive value (NPV), accuracy (Ac), positive likelihood ratio (LR+), and negative likelihood ratio (LR–). Numbers in parentheses represent the 95% Confidence Interval. *P*value from Fisher's exact test.

Comparison of LSM by cohort, METAVIR status, and fasting status. Mean LSM differs across METAVIR levels ($P<0.0001$), but does not depend on fasting status within METAVIR levels ($P=0.99$). There were no differences in mean LSM attributable to fasting by cohort ($P=0.76$). Results are from two analysis of variance models, one to assess mean LSM by cohort and fasting status, and a second to assess mean LSM by METAVIR and fasting status. An interaction term was used to investigate whether any effect in fasting status depended on the group variable (cohort or METAVIR status).

Table 3

LSM (kPa)									
Group	Fasting	N	Mean±SD	Minimum	Q1	Median	Q3	Maximum	
Calibration	Unknown	97	12.3±13.3	2.5	5.2	7.1	13.5	75.0	
Validation	Yes	44	12.2±13.7	3.2	5.6	8.2	12.0	75.0	
	No	30	14.4±14.2	3.0	6.1	10.9	16.3	75.0	
F0	Unknown	96	12.5±13.9	3.1	5.7	7.6	13.0	75.0	
	Yes	12	8.3±5.6	3.2	3.9	6.6	10.5	20.4	
	No	5	7.6±4.2	3.0	5.1	6.7	9.3	14.0	
	Unknown	30	8.7±12.7	3.1	4.8	5.9	7.6	75.0	
F1	Yes	10	6.8±3.5	3.3	4.7	5.1	10.1	14.0	
	No	12	11.3±7.4	4.2	6.1	10.9	13.0	31.6	
	Unknown	64	8.8±10.2	3.2	5.1	6.4	8.5	75.0	
	Yes	9	10.7±9.8	4.4	6.7	7.7	8.8	36.3	
F2	No	4	16.1±9.5	6.1	9.6	14.7	22.6	28.9	
	Unknown	39	11.5±14.0	2.5	5.0	6.3	8.9	68.1	
F3	Yes	5	11.0±4.1	6.5	9.4	9.5	12.2	17.5	
	No	7	19.0±25.1	4.4	6.1	9.6	17.9	75.0	
	Unknown	33	13.0±7.0	4.9	7.7	10.4	15.7	32.6	
	Yes	8	27.3±25.4	7.2	11.0	17.0	40.2	75.0	
F4	No	2	31.0±6.6	26.3	26.3	31.0	35.7	35.7	
	Unknown	27	26.0±18.3	5.9	14.6	20.8	29.9	75.0	

Table 4

Performance of cut-points to predict F3–F4 (advanced fibrosis) and F4 (cirrhosis) among n=44 fasted validation cohort subjects.

METAVIR	LSM cut-point	Cohort	N	Sn (%)	Sp (%)	PPV (%)	NPV (%)	Ac (%)	LR+	LR–
F3–F4	>8.6 kPa	Calibration	38 (39%)	79.4 (62.1, 91.3)	82.5 (70.9, 90.9)	71.1 (58.3, 81.2)	88.1 (79.2, 93.6)	81.4 (72.3, 88.6)	4.55 (2.59, 7.99)	0.25 (0.13, 0.49)
		Validation - fasting	21 (48%)	84.6 (54.6, 98.1)	67.7 (48.6, 83.3)	52.4 (38.6, 65.8)	91.3 (74.1, 97.5)	72.7 (57.2, 85.0)	2.62 (1.50, 4.59)	0.23 (0.06, 0.83)
		<i>P</i> value		1.00	0.12	0.17	1.00	0.27		
F4	>11.5 kPa	Calibration	28 (29%)	83.3 (58.6, 96.4)	83.5 (73.5, 90.9)	53.6 (40.3, 66.4)	95.7 (88.6, 98.4)	83.5 (74.6, 90.3)	5.06 (2.96, 8.67)	0.20 (0.07, 0.56)
		Validation - fasting	13 (30%)	75.0 (34.9, 96.8)	80.6 (64.0, 91.8)	46.2 (28.3, 65.1)	93.5 (81.2, 98.0)	79.5 (64.7, 90.2)	3.86 (1.78, 8.38)	0.31 (0.09, 1.04)
		<i>P</i> value		0.63	0.79	0.74	0.64	0.64		

Performance characteristics include sensitivity (Sn), specificity (Sp), positive predictive value (PPV), negative predictive value (NPV), accuracy (Ac), positive likelihood ratio (LR+), and negative likelihood ratio (LR–). Numbers in parentheses represent the 95% Confidence Interval. *P*value from Fisher's exact test.