August 12, 2016

Cystic Fibrosis Liver Disease Network (CFLD NET)

Longitudinal Assessment of Transient Elastography in Cystic Fibrosis

ELASTIC CF

Version 1.0
(ELASTIC CF)

Protocol Version 001

August 12, 2016

Steering Committee Approval Dates

07/21/2016

ELASTIC-CF development committee

Michael Narkewicz, University of Colorado, Aurora, CO
Simon Ling, Hospital for Sick Children, Toronto, Ontario
Jean Molleston, Indiana University, Indianapolis, IN
Alex Weymann, Washington University, St. Louis, MO
Wen Ye, University of Michigan, Ann Arbor, MI
John Magee, University of Michigan, Ann Arbor, MI

Design Synopsis

Title: Longitudinal Assessment of Transient Elastography in Cystic Fibrosis (ELASTIC CF)

Sponsor: National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) and CF Foundation

Study Population: All participants enrolled in the Prospective Study of Ultrasound in Predicting Hepatic Outcome in Children with Cystic Fibrosis (PUSH) longitudinal follow up at centers with Fibroscan® for transient elastography.

Primary Objective:
- To determine if transient elastography (TE), when combined with ultrasound (US) pattern characterization can improve the prediction of progression to a nodular pattern on US.

Secondary Objectives
- To confirm the feasibility of obtaining TE measurements in children with CF
- To prospectively assess whether TE data are associated with conventional laboratory markers of hepatic fibrosis
  To determine the variabliity of TE measurements taken at different sites in the same patient
  Exploratory Objectives:
- To determine if TE with fat content can improve assignment of grade in situations where there is a dichotomy of radiology grades (i.e. 2 HTG, 2NL)
- To determine if TE can predict the development of complications in children and young adults with CF with pattern of cirrhosis on US.
- To pilot the correlation of TE and hepatic fibrosis and fat content in children and young adults with CF who have a clinically indicated liver biopsy.

Inclusion criteria:
- Enrolled in the CFLD NET PUSH Study longitudinal follow up study at a center with transient elastography capability

Exclusion criteria:
- Presence of significant ascites
- Active medical device implant
- Open wound near sensor application site
- Pregnancy
- Unable or unwilling to give informed consent or assent
- Patient unable or unwilling to tolerate the TE measurement procedure

Recruitment:
- Planned minimum follow up of 24 months
- Recruitment period: November 1, 2015 to October 31, 2018

- Total sample size
  - CFLD NET: 224 subjects currently followed in CFLD NET
    - 128 normal US pattern
    - 22 homogeneous US pattern
    - 39 heterogeneous US pattern
    - 35 cirrhosis US pattern

Transient elastography is currently available at 8 centers. So current potential eligible subject numbers are shown in the table below:

	Total Subjects	NL	HMG	HTG	CIR
Centers with TE	166	90	19	33	24
3 Centers without TE	58	38	3	6	11
Total possible	224	128	22	39	35

Number of clinical centers: 8 (could expand to the full 11 based on Fibroscan availability). Washington University has the most subjects in longitudinal followup who will not be in this substudy initially (30 (21 NL, 4 HTG, 2 HMG, 3 CIR)

Visit schedule:

- TE with annual laboratory visit

Background and Goals

Noninvasive monitoring of liver fibrosis is an unmet critical need in CF liver disease. Liver biopsy is infrequently used in CF due to the patchy nature of liver involvement and is not suitable for longitudinal studies of progressive liver disease in CF. Indeed, unlike many other liver diseases, synthetic dysfunction is rare in CF liver disease. Portal hypertension due to hepatic fibrosis mediates most if not all of the complications seen in CF liver disease.

Liver involvement in CF is common, but progression to advanced liver disease (cirrhosis with portal hypertension) occurs in only 5-7% of individuals with CF. The mean age of diagnosis of advanced liver disease in CF is between 10 and 15 years of age suggesting that this is a disease of childhood. We have been conducting a prospective longitudinal cohort study to determine the utility of grayscale liver ultrasound pattern to predict the development of advanced liver disease in children with CF (PUSH: Clinical Trials: NCT01144507) for 5 years. Preliminary studies in children with CF have suggested that TE can identify children with advanced liver disease (1-4). However, to our knowledge, there has not been a prospective study of longitudinal changes in elastography in children with CF nor studies of the predictive utility of elastography for complications of liver disease in children and young adults with CF and liver disease. The addition of elastography to the ongoing PUSH study in the CFLD NET provides a unique opportunity to evaluate the

potential utility of elastography in a well characterized prospectively identified population of children with CF.

CFLD NET is a network of 11 clinical centers funded by the Cystic Fibrosis Foundation with a DCC that is funded by the NIDDK associated with the Childhood Liver Disease and Education Network (ChiLDREN). The network has been in existence since 2010 and is conducting a prospective study of the utility of standard grayscale liver ultrasound imaging to predict the risk of development of advanced liver disease as determined by ultrasound imaging. This study provides the backbone of the proposed study of elastography in CF.

While liver involvement is common in CF, estimates of advanced liver disease (cirrhosis with or without portal hypertension) suggest that only 5-7% of children with CF will develop advanced liver disease and that subsequent development in adulthood is unusual. More subtle liver involvement including hepatomegaly, persistently elevated AST, ALT or GGTP and image pattern abnormalities may occur in up to one third of CF children. The rate of progression of hepatic fibrosis and the association of stage of fibrosis and complications (mainly variceal hemorrhage in CF) is unknown. When progressive hepatic fibrosis occurs in CF, the most common complication of the subsequent portal hypertension is variceal hemorrhage and malnutrition (5). Elastrography values are higher in CF patients with varices compared to those without varices (1). In cystic fibrosis liver disease progression to ascites and hepatic synthetic failure is very uncommon. With the recent development of medications that improve or normalize the function of the basic defect in CFTR, there is an urgent need to be able to non-invasively assess changes in liver fibrosis in CF and target potentially higher risk individuals for early therapy. **A noninvasive assessment of hepatic fibrosis is desperately needed to advance the care of children with CF significant liver disease and to provide for measurements during clinical trials**. That global assessment might serve as both a predictor/descriptor of disease course but also as a critical biomarker for clinical research. FibroScan® measurement of liver stiffness has great potential to fill this void. *The underlying hypothesis of this proposal is that elastography in addition to US can improved the prediction of the development of a nodular liver on US and development of portal hypertension over time in children and young adults with CF*.

Participating Centers

There are 11 CFLD NET centers, located at prominent Children's Hospitals and medical centers in the United States and Canada with large CF clinics, who are participating in the above-mentioned on-going prospective longitudinal study that would serve as the backbone for this study. The 11 centers are (eight ChiLDREN centers are in bold):

- **Atlanta** – Emory University and Children's Healthcare of Atlanta
- Baltimore – John Hopkins University and John Hopkins Medical Center
- **Chicago** – Northwestern University and Ann & Robert H. Lurie Children's Hospital

- **Cincinnati** - Cincinnati Children's Hospital Medical Center
- **Aurora** – University of Colorado SOM and Children's Hospital Colorado
- **Houston** – Baylor Medical College and Texas Children's Hospital
- **Indianapolis** – University of Indiana and Riley Hospital for Children
- Minneapolis – University of Minnesota
- **Seattle** – University of Washington and Seattle Children's Hospital
- St Louis - Washington University and St Louis Children's Hospital
- **Toronto** - The Hospital for Sick Children and University of Toronto

Fibroscan is available through the NIH at the bolded centers. Baltimore and Minneapolis and St Louis do not have Fibroscan available at this time. If it becomes available, they will participate in this study.

The CFLD NET study is supported by the ChiLDReN Data Coordinating Center (DCC) at the University of Michigan/Arbor Research and the clinical component is funded by the CF Foundation

Study Aims and Hypotheses

- Primary Objective:
- Aim 1:
- To determine if transient elastography (TE), when combined with ultrasound (US) pattern characterization can improve the prediction of progression to a nodular pattern on US.
-
    - Hypothesis 1: TE results can differentiate children with a heterogeneous or homogeneous patterns on grayscale liver US who will progress to a nodular pattern on US

- Secondary Objectives
- Aim 2: To confirm the feasibility of obtaining TE measurements in children with CF
    - Hypothesis 2a: Valid TE measurements will be obtained in more than 90% of children with CF
    - Hypothesis 2b: To determine the variability of TE measurements taken at different liver sites in the same patient

- Aim 3: To prospectively assess whether TE data are associated with conventional markers of hepatic fibrosis and portal hypertension
    - Hypothesis 3: TE based values will correlate with conventional biomarkers of fibrosis/liver disease severity in CF: APRI, Fib-4, platelet count and spleen size

- Exploratory Objectives:
- Aim 4: To determine if TE with fat content can improve assignment of grade in situations where there is a dichotomy of radiology grades (i.e. 2 HTG, 2NL)

  - Hypothesis 4a: The combination of grayscale liver US image pattern assessment and TE values with fat content will differentiate between those individuals with dichotomous radiology grades (2HTG/2NL 2HTG/2HMG or 2HTG/2CIR) and risk of progression to a nodular pattern on US.
  - Hypothesis 4b: Higher fat content will be associated with a lower risk of progression to a nodular pattern on US

-
- Aim 5: To determine if TE can predict the development of portal hypertension and its complications in children and young adults with CF and a nodular pattern on US.
  - Hypothesis 5: Among those children with a nodular pattern on US, TE results will correlate with clinical findings of portal hypertension (splenomegaly, thrombocytopenia, variceal hemorrhage and/or ascites)
- Aim 6: To pilot the correlation of TE and hepatic fibrosis and fat content in children and young adults with CF who have a clinically indicated liver biopsy.
  - Hypothesis 6: TE assessment of liver stiffness and fat content will correlate with steatosis and fibrosis staging on liver biopsy in CF

Inclusion/Exclusion Criteria

Inclusion criteria:

- Participants enrolled in CFLD NET PUSH study in longitudinal follow up at centers with Fibroscan available (currently 8/11 centers)
- Entry criteria for that study were:
  - CF as determined by sweat chloride >60 meq/l
  - Pancreatic insufficiency
  - Age 3-12 years old at entry
  - For entry into the longitudinal follow up subjects were in one of two groups
    - A screening US pattern of nodular liver (CIR), heterogeneous increased echogenicity (HTG) or homogeneous increased echogenicity (HMG)
    - A screening US pattern of normal (NL) matched to a HTG subject (2 NL:1HTG) by age, center and pseudomonas status

Exclusion criteria:

- Exited from the PUSH Study
- Unable / unwilling to sign consent

Recruitment, enrollment and follow-up

- Planned minimum follow up 2 years
- Recruitment period: November 1, 2016 to October 31, 2019
- Total sample size 200 subjects assuming a 90% success

US pattern	Subjects in Study at	Potentially available

	centers with TE	subjects at centers currently without TE
NL	90	38
HTG	33	6
HMG	19	3
CIR	24	11

Study Procedures

The research protocol will encompass the prospective longitudinal analysis of FibroScan measurements of liver stiffness in children with CF who are enrolled in the PUSH study. Visits will occur annually for at least two years, therefore there will be a minimum of three assessments, a baseline and at least two annual follow up visits.

At each visit the comprehensive clinical data and biosample collections will occur according to the PUSH protocol. Research US is performed every 2 years. FibroScan measurements will be done using a standardized protocol at each of these visits by a designated CFLD NET investigator with a specialized CRF completed for the Fibroscan measurements.

One or two investigators will be trained at each site to perform TE measurements to ensure consistent and standardized acquisition of complete data. Fasting will be specified for these procedures. While fasting requirements can significantly complicate pediatric-based studies, adult data suggest that fasting can affect elastography values (6, 7). We have been successful with fasting for the Doppler US evaluations in this study and have processes in place to successfully do studies in fasted individuals. Thus we will plan for a minimum of a 4 hour fast for the TE. The exam time is estimated to be 10-20 minutes. No lower limit age restrictions are incorporated in this protocol to afford an accurate assessment of feasibility in children of all ages (the youngest subject in the study is currently 5 years old). No sedation is needed for TE assessments.

Schedule of Visits

Procedure	Baseline	12 months	24 months
Informed Consent	X		
TE Measurement	X	X	X
TE CRF	X	X	X
Clinical data per PUSH	X	X	X
Biospecimens per PUSH	X	X	X
Research US per PUSH (occurs every 2 years, will vary on entry phase)	US will be performed on the ongoing schedule. TE will be timed to coincide with annual bloodwork and clinical visit.		

Data Collected
Clinical and laboratory data including laboratory values, physical examination, medical history, ultrasound, and biosamples will be collected per the PUSH protocol. Additional liver biopsy results will also be collected for patients enrolled in this proposed ancillary study.

Transient Elastography (FibroScan®) measurements

Up to 6 personnel will be trained at each site to perform FibroScan™ measurements to ensure consistent and standardized acquisition of complete data. While we will focus on one or two personnel involved in PUSH at each center, FibroScan™ will be available at ChiLDReN centers for multiple studies with more investigators trained. By allowing up to 3 investigators per site, we will reduce the likelihood of missing any measurements on eligible subjects. Training will take place at each site by a designated trainer from EchoSens.

Patients will be fasted for a minimum of 4 hours. No sedation will be administered for these FibroScan™ assessments. The exam time is estimated to be 10 to 20 minutes.

The thoracic perimeter of the patient will be measured and recorded. The thoracic perimeter value will determine selection of the probe as follows: >70cm M-probe, >45cm S2 probe, ≤ 45 cm S1 probe. The patient will be positioned in the dorsal decubitus position with the right arm in maximal abduction. The operator will sit on a chair on the right side of the patient facing both the patient's chest and the screen of the device. A small amount of coupling gel is applied to the right chest wall. The probe is placed on the chest wall, in the mid-axillary line, over the right lobe of the liver (identified by percussion) between the ribs, angled towards the middle of the parenchyma and away from the liver border. The probe is adjusted until a liver portion, free of large vascular structures, is identified. The probe is kept perpendicular to the skin and a firm amount of pressure is applied. When all these conditions have been met and an ideal window of liver tissue is identified on the device screen, the button on the probe is pressed, without changing the probe position. The device records and displays the validity of each measurement based on standardized criteria determined by Echosens. Repeated measurements are performed until 10 valid values are obtained (maximum of 16 attempts)

A second measurement site will then be identified that is one intercostal space superior or inferior to the initial measurement site. The TE measurement will be repeated at this second site, as described above.

After the end of the examination the gel is removed from the patient's chest wall with a soft tissue. Gel is also removed from the probe with a soft towel and it is then disinfected with a solution containing quaternary ammonia. The report is printed

and a non-identifying study ID label is applied. The report will be placed in the research binder and not in the clinical chart.

FibroScan™ is based on vibration controlled TE at 50Hz. FibroScan measures 2 parameters:

- "Liver stiffness" quantifies liver fibrosis and is measured in kPa (median of 10 subsequent valid measurements and are deemed acceptable if the ratio of interquartile range and median is <30% and success rate is >60%, meaning 10 valid measurements are obtained within 16 attempts).
- "Controlled Attenuation Parameter (CAP)" quantifies liver steatosis and is measured in dB/m (median of 10 subsequent valid measurements).

In addition quality control data are collected:

- Invalid measurements and success rate
- Number and list of valid measurements
- Inter quartile range (IQR) (kPa or dB/m) of all valid measurements within the examination (reflects the dispersion of stiffness or CAP measurements)
- IQR/med. (%) Indicates the IQR/median ratio and should remain as low as possible to ensure reliable results (goal < 30%)

Study withdrawal/discontinuation

The participant and/or participant's family may withdraw from the study at any time. The study investigator can withdraw the participant from the study at any time if that is felt to be in the best interest of the participant and/or their family. If a participant in the longitudinal follow up study meets an endpoint for the end of the PUSH study (liver transplantation or death) they will have met the end of this study.

Statistical and Design Considerations

Based upon interest and involvement of the subjects in PUSH, we anticipate all subjects currently available (168) will enroll. If TE becomes available at the 3 centers without TE, then up to an additional 60 subjects would be available to enroll. TE would be available from all eligible US pattern subjects.

Hypothesis 1: TE results can differentiate children with a heterogeneous or homogeneous pattern on grayscale liver US who will progress to a nodular pattern on US

Analysis:
To test whether TE, when combined with US pattern characterization can improve the prediction of progression to a nodular pattern on US, we will build two discrete survival models using logistic regression. In the first model, greyscale US consensus grade will be used to predict prediction to nodular pattern. In the second model, we will add the predictor liver stiffness measured by TE. If the second model significantly increases the area under the receiver operating characteristic curve

(AUROC) comparing to the first model, we will conclude that TE can improve the prediction of progression to a nodular pattern on US. All subjects graded as NL, HMG, or HTG at the time this ancillary study starts will be included in the analysis.

Power calculations:
The estimated 2-4 year progression rate is ~40% in HTG subjects. Thus of the 33 available with HTG pattern, 10-13 would be predicted to progress to CIR. However, as the study is in progress, ~20% have progressed so the risk of progression may be lower. We choose to make a conservative assumption in our power calculation: 20% of HTG subjects will progress to CIR pattern, and 2% of NL or HMG subjects will progress to CIR pattern. In addition, we assume the liver stiffness measure follow a lognormal distribution with location =0 and scale parameter =0.5 (i.e. median=1, Q1=0.71, Q2=1.4). With 142 subjects (approximately 63% NLs, 23% HTGs, and 13% HMGs) we will have 80% power to detect an odds ratio of 4 for a 1 unit increase in the liver stiffness measure. (Caveat: Given that we do not have any preliminary data of TE measures in CF subjects, the results of this power calculation only give us a general guidance).

Hypothesis 2a: Valid TE measurements will be obtained in more than 90% of children with CF

Analysis:
For hypothesis 2a, we will estimate the proportion of subjects in whom a valid transient elastography measurement can be obtained. At each site, all measurements will be performed by one of the trained examiners. The results will be reported as the median of 10 valid measurements and will be accepted as valid if the ratio of the interquartile range (IQR) and median was < 30% and the success rate was > 60% (8, 9). Per Goldschmidt 2013 (9)we will define two measures of the feasibility of transient in our population: "technically possible" and "acceptable quality." The proportion of subjects with a "technically possible" FibroScan™ is defined as the number of subjects with at least 10 FibroScan™ measurements obtained divided by the number assessed. The proportion of subjects with FibroScans™ of "acceptable quality" is defined as the number of subjects with FibroScan™s with the ratio of the interquartile range and median of the 10 measurements <30%, divided by the number assessed. The proportions and their 95% confidence intervals will be provided using the Wald method. We will do a stratified (separate) analysis by <8 years of age and >8 years of age. There will be the opportunity to compare this data to children with other liver diseases in the ChiLDReN FORCE study.

Hypothesis 2b: To determine the variability of TE measurements taken at different liver sites in the same patient

Analysis

Variability of TE measurements at a single liver site will be quantified by the ratio of the interquartile range and the median (RIM). Absolute difference in RIM between the two liver measuring sites in the same patient will be calculated. Distribution of this absolute difference will be studied using kernel density estimation to identify whether the distribution has more than one mode (whether these are heterogeneous groups according to difference in variabilities between sites in the same patient). We will also study whether this RIM is associated with the median of the stiffness measurements in the same patient.
Hypothesis 3: TE based values will correlate with conventional biomarkers of fibrosis/liver disease severity in CF: APRI, Fib-4, platelet count and spleen size

Analysis: Graphic method will be used to explore relationship between TE based values and conventional biomarkers. Pearson or Spearman correlation will be calculated.

Exploratory analyses:

Hypothesis 4a: The combination of grayscale liver US image pattern assessment and TE values with fat content will differentiate between those individuals with dichotomous radiology grades (2HTG/2NL 2HTG/2HMG or 2HTG/2CIR) and risk of progression to cirrhosis.

Analysis:
ANOVA or Kruscal-Wallis test, whichever is appropriate, will be used to compare TE measures between the three groups.

Hypothesis 4b: Higher fat content will be associated with a lower risk of progression to cirrhosis

To test whether subjects with a high TE determination of fat content and heterogeneous pattern on US will not progress to an US pattern of cirrhosis (Hypothesis 5b), we will add the TE measure of fat content to the second model for testing hypothesis 1 (i.e. this model will include three predictors: greyscale US, liver stiffness, and fat content). The hypothesis can tested through testing the regression coefficient for the fat content variable. A significant negative regression coefficient will support our hypothesis. All subjects graded as NL, HMG, or HTG at the time this ancillary study starts will be included in the analysis.

Hypothesis 5: Among those children with a nodular pattern on US, TE results will correlate with clinical findings of portal hypertension (splenomegaly, thrombocytopenia, variceal hemorrhage and/or ascites)

Analysis:
For this analysis we will include all subjects with a nodular US pattern (CIR). The primary outcome will be a composite outcome defined as occurrence of any of the following three endpoints:

Hypersplenism: platelets <150,000
Variceal hemorrhage
Splenomegaly as indicated by a palpable spleen
Ascites as clinically detected ascites

A discrete survival analysis using logistic regression will be used to test whether transient elastography results are associated with risk of developing liver cirrhosis complications. The predictor will be most recent TE results.

Power calculation:
Previous literature has reported mean a rate of variceal hemorrhage between 5 and 10% per year with CF and cirrhosis. There is no data for rates of development of splenomegaly or thrombocytopenia in CF with a nodular US pattern. With currently 33 subjects with a nodular US pattern and 90% successful rate of TE, we anticipate 3 to 6 subjects (10% ~20%) among 30 subjects developing one of the endpoints in 2 years. We assume the true difference in population mean TE measurements is 19 kPa between subjects with and without portal hypertension; we assume the SD is 22.0 kPa and 6.0 kPa for subjects with and without portal hypertension, respectively. Our calculation shows that using 2-sided likelihood ratio Chi-square test (Type I error rate 5%) there will be 80% power to detect odds ratio 4 ~ 5 per 10kPa increase in TE results.

Hypothesis 6: TE assessment of liver stiffness and fat content will correlate with steatosis and fibrosis staging on liver biopsy in CF

Analysis: ANOVA or Kruscal-Wallis test, whichever is appropriate, will be used to compare TE measures between groups based on steatosis and fibrosis staging on liver biopsy in CF.

Human research participant issues

Institutional Review Board Approval

Local institutional review board approval will be obtained for the study. IRB approvals will be centrally collated at the Data Coordinating Center.

Standard of Care

Standards of care for the management of liver disease at each of the CFLD NET sites will be followed. At present there are no clinical standards for interventions based on TE-measured liver stiffness for pediatric CF patients and thus there are no expected interventions based solely on TE measurements.

Consent

Informed consent and assent (when appropriate according to local standards) will be obtained prior to any research activities. A common template for

the informed consent form will be used by all of the centers, modifying the content or format as necessary to meet the requirements of their respective institutional human subjects committees.

Privacy/Confidentiality Issues

All subjects have a unique CFLD NET research study ID that is generated by the DCC. While the study is ongoing, the clinical site will maintain a link between the research study number and the subject's identity. However, this information will not be contained in any data file that is transmitted to the DCC. When the study ends, each clinical site will destroy the linkage between the research study number and the subject's identity.

Adverse Event Reporting

Given the observational nature of the study it is anticipated that study related adverse events will be very unlikely. The only serious adverse events related to the performance of this study are those related to obtaining the TE measurements. Adverse event reporting will be formally specified in the final protocol and data safety monitoring plan.

Data Safey Monitoring Board (DSMB)

Study oversight will be provided by the CFLD NET PUSH DSMB established by the NIDDK.

Visit Schedule

Annual assessments for a minimum of two years

References

1. Malbrunot-Wagner AC, Bridoux L, Nousbaum JB, Riou C, Dirou A, Ginies JL, Maurage C, et al. Transient elastography and portal hypertension in pediatric patients with cystic fibrosis Transient elastography and cystic fibrosis. J Cyst Fibros 2011;10:338-342.
2. Menten R, Leonard A, Clapuyt P, Vincke P, Nicolae AC, Lebecque P. Transient elastography in patients with cystic fibrosis. Pediatr Radiol 2010;40:1231-1235.
3. Rath T, Menendez KM, Kugler M, Hage L, Wenzel C, Schulz R, Graf J, et al. TIMP-1/-2 and transient elastography allow non invasive diagnosis of cystic fibrosis associated liver disease. Dig Liver Dis 2012;44:780-787.
4. Witters P, De Boeck K, Dupont L, Proesmans M, Vermeulen F, Servaes R, Verslype C, et al. Non-invasive liver elastography (Fibroscan) for detection of cystic fibrosis-associated liver disease. J Cyst Fibros 2009;8:392-399.
5. Debray D, Lykavieris P, Gauthier F, Dousset B, Sardet A, Munck A, Laselve H, et al. Outcome of cystic fibrosis-associated liver cirrhosis: management of portal hypertension. J Hepatol 1999;31:77-83.
6. Arena U, Lupsor Platon M, Stasi C, Moscarella S, Assarat A, Bedogni G, Piazzolla V, et al. Liver stiffness is influenced by a standardized meal in patients with

chronic hepatitis C virus at different stages of fibrotic evolution. Hepatology 2013;58:65-72.
7. Mederacke I, Wursthorn K, Kirschner J, Rifai K, Manns MP, Wedemeyer H, Bahr MJ. Food intake increases liver stiffness in patients with chronic or resolved hepatitis C virus infection. Liver Int 2009;29:1500-1506.
8. Abenavoli L, Corpechot C, Poupon R. Elastography in hepatology. Can J Gastroenterol 2007;21:839-842.
9. Goldschmidt I, Streckenbach C, Dingemann C, Pfister ED, di Nanni A, Zapf A, Baumann U. Application and limitations of transient liver elastography in children. J Pediatr Gastroenterol Nutr 2013;57:109-113.