EVALUATION OF LIVER STIFFNESS MEASUREMENT BY FIBROSCAN AS COMPARED TO LIVER BIOPSY FOR ASSESSMENT OF HEPATIC FIBROSIS IN CHILDREN WITH CHRONIC HEPATITIS C

By

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Abstract

The study evaluated liver stiffness measurement (LSM) using non-invasive transient elastography (TE) in comparison with liver biopsy for assessment of hepatic fibrosis in children with chronic hepatitis C (CHC). Thirty children (mean age 10.13±3.4 years) with CHC were subjected to histopathological assessment of liver biopsy specimens using METAVIER score and LSM using TE (FibroScan) as well as appropriate laboratory investigations. The results showed a highly significant stepwise increase of the mean liver stiffness values with increasing histological severity of hepatic fibrosis with the highest level detected in patients with stage F4 "cirrhosis" and significant differences for F3 and F4 vs. other fibrosis stages. There were significant positive correlations between LSM and several parameters of activity and progression of the chronic liver disease including METAVIER fibrosis stages (r=0.774, p=0.0001), necroinflammatory activity grades, AST, ALT, total serum bilirubin, prothrombin time and Child-Pugh grades as well as biochemical serum fibrosis markers (Fibrotest, Actitest, AST-to-platelet ratio index, Forns index and hyaluronic acid). The variables significantly negatively associated with the LSM were platelets count and serum albumin. The highest predictive performance of LSM was detected for stage F4 "cirrhosis", followed by F3 "advanced fibrosis" where accuracy of (96.7%, 85.3%) and AUROC of (1.00, 0.815) were obtained for these fibrosis stages at cutoff values of 9.5 and 12.5 kPa, respectively. The negative predictive values to exclude advanced fibrosis and cirrhosis at these cutoffs were high, whereas positive predictive values were modest.

Key words: Liver stiffness; Transient elastography; Liver biopsy; Chronic hepatitis C; FibroScan; Children.

Introduction

Hepatitis C virus (HCV) infection is a main cause of chronic liver diseases (CLDs) (Seeff *et al.* 2002) and the prognosis of patients with CLDs is de termined primarily by the extent and progression of hepatic fibrosis. Hepatic fibrosis is a wound-healing response to chronic liver injury, which may lead to cirrhosis and hepatocellular carcinoma

(Benvegnu *et al,* 2004). Follow-up of appearance and progression of liver fibrosis is indicated to initiate antiviral treatment and anticipate the possible necessity for liver transplantation. Percutaneous liver biopsy followed by conventional histological analysis remains the standard method for assessing fibrosis. But, liver biopsy is an invasive and painful procedure that can have life-threatening complications in both adults and children, which may limit its acceptance by patients and make repetition difficult for difficult-to-treat patients (Lachaux *et al.* 1995, Scheimann *et al.* 2000). Also, the accuracy of liver biopsy in assessing fibrosis can be questioned because of sampling error and intra- and interobserver variability that may lead to over- or understaging of fibrosis (Bedossa *et al.* 2003, Colloredo *et al.* 2003, Regev *et al.* 2002). Therefore, there is a need for alternative noninvasive methods for evaluating the stage of hepatic fibrosis and its severity in liver disease (Fontana *et al.* 2002). Proposed approaches, including routine biochemical and hematological tests, and surrogate serum fibrosis markers as Fibrotest and aspartate transaminase to platelets ratio index (APRI), are not accurate enough, not routinely available, or not validated for children (Imbert-Bismut *et al.* 2001, Forns *et al.* 2002, Wai *et al.* 2003).

Among other noninvasive methodologies, transient elastography (Fibro-Scan®; Echosens, Paris, France) has been validated first in adult patients with HCV infection. Transient elastography (TE) is a novel, rapid, non-invasive, and reproducible method for measuring liver stiffness that determines the organ elasticity by measuring the velocity of a low-frequency shear wave going through the liver. The harder the tissue, the faster the shear wave propagates (Sandrin *et al.* 2003). Several studies have demonstrated that liver stiffness measurement (LSM) is closely related to fibrosis stage as assessed by liver biopsy in adult patients with chronic hepatitis C (Saito *et al.* 2004, Ziol *et al.* 2005, Castera *et al.* 2005, Talwalkar *et al.* 2007). Encouraging results concerning feasibility and accuracy of TE in liver fibrosis prediction in a cohort of miscellaneous pediatric CLDs were provided by De Le´dinghen *et al* (2007) who concluded that there is a need for other studies to evaluate FibroScan performance in children according to each cause of CLDs. But, few studies have examined the evaluation of liver fibrosis using non-invasive TE (Fibro Scan) in children with CLDs including chronic HCV ones (Breton *et al.* 2009). Thus, the aim of this prospective study was to evaluate the diagnostic performance of LSM using non-invasive TE (FibroScan) in comparison with liver biopsy for assessment of hepatic fibrosis in children with chronic HCV infection and correlation of LSM with clinicopathological parameters and some serum fibrosis markers of CLDs.

Patients, Materials and Methods

This prospective study included thirty (30) children suffering from chronic hepatitis C (CHC) who were selected from those admitted to the Hepatology

Unit, Pediatric Department, Tanta University Hospitals from April 2012 to June 2013. Appropriate laboratory examinations, liver biopsy (LB) and LSM using non-invasive TE (FibroScan) were performed for all children after obtaining written informed consent from their parents. Chronic hepatitis C was defined by the presence of anti-HCV antibodies and detectable serum HCV-RNA by PCR (>50 IU/ml) for over 6 months. Subjects with one or more of the following conditions were excluded: co-infection with fascioliasis, schistosomiasis or human immunodeficiency virus and/or hepatitis B or delta virus; other causes of CLDs; or liver surgery or transplantation. Patients with ascites or obesity, failure to obtain LSM, uninterpertable liver biopsy or children less than 2 years old, were excluded. The study was approved by the Postgraduate Clinical Research and Ethics Committee of Tanta Faculty of Medicine.

Percutaneous liver biopsy (LB) was performed in all subjects by an experienced hepatologist, after an overnight fast, using a disposable automatic core biopsy 18-Gauge needle (Auto-Vac biopsy needle, Germany) under conscious sedation and ultrasound guidance. The length of each liver fragment and the number of portal tracts were recorded and only patients with LB length ≥15 mm and/or at least 10 portal tracts were included. Liver specimens were fixed in formalin and embedded in paraffin. Sections measuring 4 mm were stained with Hematoxylin-Eosin (H&E) and with Masson trichrome, and reticulin stains for evaluation of fibrous tissue. All liver specimens were analyzed by an experienced pathologist blinded to results of Fibro Scan and biochemical fibrosis markers. Histological features were analyzed using METAVIR group scoring system that consists of 5 stages according to the architectural features of the portal fibrosis: F0=no fibrosis, F1=portal fibrosis without septa, F2=portal fibrosis and few septa, F3=numerous septa without cirrhosis, and F4=cirrhosis. When there was a disparity between 2 adjacent stages, scores were allocated for the more advanced stage in all children. Significant fibrosis was defined by presence of F2, F3, or F4 METAVIR stage and the presence of F3 or F4 stages characterized advanced fibrosis. The activity grade including the intensity of necroinflammation was scored as: A0, no histological activity; A1, mild; A2, moderate; and A3, severe (METAVIR Cooperative Study Group 1994, Bedossa et al. 1996).

Transient elastography examinations were performed by a single experienced operator prior to LB, on the same week of the procedure. Measurements were performed by using the standard technique, as previously described elsewhere (Sandrin et al, 2003, Castèra et al, 2005, De Le´dinghen et al, 2007). Only patients with at least 10 valid measurements, with an interquartile range of less than 30% of the median stiffness expressed in kilopascal (kPa), and with at least 60% success rate were included in the final analysis. According to previous studies on HCV infection (Ziol et al, 2005, Castèra et al. 2005), the following cut-off values

were used: 4.9 kPa for mild fibrosis (F≥1), 7.1 kPa for significant fibrosis (F≥2), 9.5 kPa for advanced fibrosis (F≥3) and 12.5 kPa for cirrhosis (F=4).

Laboratory tests: stool analysis, CBC, liver and renal function tests, biochemical serum fibrosis markers (Fibrotest, Actitest, APRI, Forns index and Hyaluronic acid), also virological markers were assessed prior to LB, on the same week that LB and TE were performed. Anti-HCV antibodies were determined by a commercial ELISA Kits and HCV-RNA was determined by PCR.

Biochemical serum fibrosis markers: Fibrotest and Actitest were chosen because they are the most validated biochemical scores of fibrosis (Imbert-Bismut *et al.* 2001). Forns and APRI indices are simple and free tests based on the standard blood parameters included in standard biological liver function assessment (Forns *et al.* 2002, Wai *et al.* 2003)**.** Hyaluronic acid (HA) measurement may be helpful in differentiating non-cirrhotic from cirrhotic liver, monitoring liver function and evaluating the extent of liver fibrosis. Hyaluronic acid was measured by commercial ELISA Kits (Khan *et al,* 2007).

Statistical analysis: Continuous variables were compared using Student's *t*-test, the Mann-Whitney test, or the Kruskal–Wallis test when appropriate. Categorical variables were compared using the Chi-square test or Fisher's exact test. Statistical analysis was performed by SPSS software version 17.0 (SPSS Inc., Chicago, IL, USA). Diagnostic performance of TE in children with CHC was assessed by comparison with liver histology and by measuring area under receiver-operating characteristics curve (AUROC). ROC curve comparisons were performed using the medcalc software package version 9.3 (MedCalc Software, Mariakerke, Belgium), which employs calculation of AUROC and 95% confidence intervals. Commonest used index of accuracy is AUROC, with values close to 1.0 indicating a high diagnostic accuracy. Diagnostic accuracy was evaluated by comparing sensitivity, specificity, positive and negative predictive values (PPV and NPV respectively) of TE measurement to predict the absence or presence of mild fibrosis, significant fibrosis, advanced fibrosis and cirrhosis by using the appropriate cut-offs points, as described above. The probability of a true positive (sensitivity) and true negative (specificity) assessment was determined for selected cutoff values. A patient was assessed to be positive or negative according to whether liver stiffness value was greater than, less than, or equal to a given cutoff value. Correlation between variables was evaluated using the Spearman correlation coefficient. *P* values of less than 0.05 were considered significant.

Results

Liver stiffness values in relation to severity of liver fibrosis (Tab. 1), there was a highly significant stepwise increase of the mean liver stiffness (LS) values (p<0.001) with increasing histological severity of hepatic fibrosis with the highest value detected in stage F4 (cirrhosis) as follow: 3.33±1.00 kPa for F0, 5.46±1.09 kPa for F1, 8.13±0.92

kPa for F2, 11.50±2.16 kPa for F3 and 40.28±18.06 kPa for F4. Significant differences in LSMs were found when patients with stage F3 and those with F4 were compared to patients with other fibrosis stages (p<0.001). For pairwise comparisons of other fibrosis stages (F0, F1, F2), LSMs did not differ (P> 0.05). To represent the LSMs according to the histologic fibrosis grading, box plots were prepared (Fig. 1A). A steady stepwise increase of the LS values was observed with increasing severity of hepatic fibrosis. These findings indicate that the more advanced the histological fibrosis stages and liver disease, the stiffer the liver tissue.

Liver stiffness values in relation to severity of necroinflammatory activity: Mean LS values significantly increased (p<0.001) in accordance with increased severity of activity grading of chronic HCV infected children as follow: 6.61±6.82 kPa for A1, 11.14±8.15 kPa for A2, 30.64±26.25 kPa for A3 (Tab.1, Fig.1B).

Table 1: Relationship between LSM and histological parameters

Histological parameters	Liver stiffness values (kPa)			Kruskal-Wallis test	
	Median	Range	Mean±SD	χ^2	P-value
Fibrosis stages					
F0 (n=5)	3.70	2.2-4.1	3.33±1.00	26.198	0.0001*
F1 (n=10)	6.10	4.0-6.7	5.46±1.09		
F2 (n=7)	8.10	6.9-9.3	8.13±0.92		
F3 (n=3)	10.30	10.2-14	11.50±2.16		
F4 (n=5)	32.90	19.9-59.4	40.28±18.06		
Mann-Whitney U test P value	P <0.001* for F4 vs. other stages P <0.001* for F3 vs. F0,F1,F2 stages				
Activity grades					
A1 (n=14)	4.60	2.2-29.9	6.61±6.82	17.61	0.0001*
A2 (n=11)	8.10	6.1-32.9	11.14±8.15		
A3 (n=5)	14.00	10.2-59.4	30.64±26.25		
Mann-Whitney U test P value	A1 vs. A2, P=0.013* A1 vs. A3, P=0.0001* A2 vs. A3, P=0.003*				

*Significant (P<0.05)

There were significant positive correlations (Tab. 2) between LSM and both of fibrosis stages (Fig. 2A) and necroinflammatory activity grades (Fig. 2B) in children with CHC. The other variables significantly positively associated with the FibroScan values were AST, ALT, total serum bilirubin, prothrombin time and Child Pugh grades as well as noninvasive biochemical serum fibrosis markers including Fibrotest, Actitest, APRI, Forns index and hyaluronic acid (all P <0.05). The variables significantly negatively associated with FibroScan values were platelets count and serum albumin level (P <0.05).

These findings indicate that LSM by FibroScan reflects activity and progression of the chronic liver disease. There was progressive elevation of diagnostic accuracy of LSM (Tab. 3) with increasing stage of liver fibrosis as follow: for F≥1 (mild fibrosis) sensitivity of 71.4 %, specificity of 65.2% and accuracy of 60.7% were detected at cutoff value of 4.9 kPa; for F≥2 (significant fibrosis) sensitivity of 100%, specificity of 81.5% and accuracy of 73.3% were detected at cutoff value of 7.4kPa; for F≥3 (advanced fibrosis) sensitivity of 100%, specificity of 81.5% and accuracy of 85.3% were detected at cutoff value of 9.5 kPa; for F=4 (cirrhosis) 100% sensitivity, 96.0% specificity and 96.7% accuracy of were detected at cutoff value of 12.5 kPa.. These results indicate that TE is an accurate tool for the prediction of advanced liver fibrosis and cirrhosis. The NPVs to exclude F3 or greater disease and cirrhosis were high (100%), whereas PPVs were modest (60.0%, 83.3%), at cutoff values of 9.5 & 12.5kPa, respectively. So, LSM main value was to exclude advanced fibrosis and cirrhosis as a screening test.

Table 2: Correlation between LSM and histopathological parameters as well as clinicolaboratory variables and serum fibrosis markers

Variables	liver stiffness measurement (LSM) (n=30)	
	r	P
Histopathological parameters		
Fibrosis stages	0.774	0.0001*
Activity grades	0.539	0.002*
Clinicolaboratory variables		
Age (ys)	0.337	0.068
Height (m)	0.315	0.288
Weight(kg)	0.115	0.388
Red blood cells (10^6/ul)	0.214	0.255
Hemoglobin (gm/dl)	0.175	0.354
Platelets (x10^3/ul)	-0.640	0.001*
White blood cells (x10^3/ul)	-0.260	0.165
Serum ALT (IU/L)	0.530	0.035*
Serum AST (IU/L)	0.521	0.035*
Total serum bilirubin (mg/dl)	0.547	0.025*
Direct serum bilirubin (mg/dl)	0.123	0.519
Total serum proteins (gm/dl)	-0.367	0.05
Serum albumin(gm/dl)	-0.619	0.001*
Prothombin time (PT) (sec.)	0.611	0.001*
INR	0.242	0.198
Serum Creatinine (mg/dl)	-0.191	0.312
Child-Pugh grades	0.655	0.001*
Biochemical fibrosis markers		
Fibrotest	0.773	0.001*
Actitest	0.542	0.005*
APRI index	0.564	0.03*
Forns index	0.762	0.001*
Hyaluronic acid (ug/l)	0.941	0.0001*

*Significant (P<0.05); APRI, AST-to-platelet ratio index; ALT and AST, Alanine and Aspartate aminotransferases; r=Correlation coefficient; INR, international normalization ratio.

Table 3: Predictive performance of LSM for liver fibrosis stages.

Predictive values of liver stiffness	F ≥1 (F0 vs. F1–4)	F ≥ 2 (F0, 1 vs. F2–4)	F ≥ 3 (F0–2 vs. F3-4)	F = 4 (F0–3 vs. F4)
Cutoff value (kPa)	4.9	7.4	9.5	12.5
Sensitivity (%)	71.4	100	100	100
Specificity (%)	65.2	81.5	81.5	96.0
PPVs (%)	38.5	37.5	60.0	83.3
NPVs (%)	88.2	100	100	100
Accuracy (%)	60.7	73.3	85.3	96.7

NPVs, negative predictive values; PPV**s,** positive predictive values

The area under curve (AUROC) for liver stiffness (Fig. 3) was 0.031 when discriminating between F0 vs. F1-4 (F≥1); 0.652 when discriminating between F0-1vs.F2-4 (F≥2); 0.815 when discriminating between F0–2 vs. F3-4 (F≥3); and 1.0 when discriminating between F0-3 vs.F4 (F=4). These results indicate that the predictive performance of TE show progressive elevation with increasing stage of fibrosis with the highest performance in cirrhosis followed by severe fibrosis, then significant fibrosis. Thus, TE is an accurate tool for the prediction of advanced liver fibrosis and cirrhosis.

Discussion

The results of this study confirmed that TE measurement is an accurate tool for the non-invasive diagnosis of liver fibrosis in children with CHC.

The current study showed that there was a highly significant stepwise increase of LS values with increasing histological severity of hepatic fibrosis with the highest level in patients with F4 (cirrhosis) and a significant positive correlation was observed between LSM & METAVIR fibrosis stages (r=0.774, p <0.001). These results are in harmony with that reported by several prior studies on patients with CLDs of various causes including those with CHC, both in children (De Lédinghen et al, 2007; Breton et al, 2009; Fitzpatrick et al, 2013) and adults (Foucher et al, 2006; Coco et al, 2007; Ogawa et al, 2007, Lupsor et al, 2008). As in this study, those authors found a good agreement between FibroScan results and fibrosis stages and TE values significantly increased with higher fibrosis stages. These findings indicate that, the more advanced histopathological fibrosis stages and the liver disease, the stiffer the liver tissue.

These observations that were reported in different studies in patients with CLDs are reliable, because the stiffness of a tissue largely depends on the molecular building collagen blocks (septa) and on the microscopic structural organization of these blocks (Fung, 1993). However, several authors have reported that LSM values may be falsely high reaching the cirrhotic range even in the absence of fibrosis on histology in patients with acute flares of viral hepatitis (Sagir et al. 2008), severe hepatic necroinflammation (Arena et al, 2008), extrahepatic cholestasis

(Millonig *et al,* 2008), hepatic congestion (Millonig *et al*, 2010), hepatic amyloidosis (Janssens *et al,* 2010) and recent food intake within 60 min (Mederacke *et al,* 2009). However, patients with such conditions were not included in the present study. Thus, in clinical practice, each LSM has to be interpreted taking into account the concurrent clinical presentation of the patient.

The current study showed that significant differences in LS values were found when patients with stage F3 and those with F4 were compared to patients with other fibrosis stages whereas for pair-wise comparisons of other fibrosis stages, LSMs did not differ. Thus, there was an overlap in the values determined by TE between fibrosis stages F0 and F1, and between fibrosis stages F1 and F2. This result suggests that TE has utility in distinguishing between no fibrosis and advanced fibrosis or cirrhosis, but has limited value for detecting mild or moderate fibrosis and thus cannot be definitively used to discriminate between early fibrosis stages. These findings are in harmony with that reported by De Lédinghen *et al.* (2007) and Breton *et al.* (2009) in children with CLDs of varying etiology, as well as Ziol *et al.* (2005) in adult patients with CHC who found that fibrosis scores by FibroScan below 8.8 kPa, were considered as small as possible without distinction between F0 and F1or F2.

It is well known that the accumulation of extracellular matrix is the major determinant of liver stiffness. However, it is conceivable that cellular edema and necroinflammatory changes could also influence TE measurement. This concept is supported by prior studies that observed increasing values of liver stiffness in the presence of high grades of necroinflammatory activity (Coco *et al.*2007, Arena *et al* 2008, Sagir *et al.* 2008, Lupsor *et al.* 2008, Myers *et al.* 2010, Cardoso *et al.* 2012). In agreement of these studies, this study showed a steady increase of TE values in parallel with the degree of necroinflammatory activity in HCV infected children and a significant positive correlation was observed between the increasing TE values and the degree of necro-inflamatory activity of liver histology (r=0.539, p=0.002). But, Fitzpatrick *et al.* (2013) reported that only severe inflammation on biopsy had a statistically significant relation with TE and lesser degrees of inflammation did not appear to affect TE reading a pediatric cohort with CLDs. In a study by Fraquelli *et al.* (2011), severe/moderate necro-inflammatory activity was independently associated with fibrosis overestimation in HCV subjects. Nevertheless, the comparison of diagnostic accuracies of TE in subjects with different grades of necroinflammatory activity (expressed by different ALT levels) as assessed by AUROC analysis, showed no influence of ALT activity on the overall performance of TE in predicting significant fibrosis, advanced fibrosis and cirrhosis (Cardoso *et al,* 2012). This discrepancy between results might be because of different populations, different etiologies and number of patients of CLDs studied as well as differences in the severity of CLDs in the studied cohorts which had

an influence on the results (Friedrich-Rust *et.al,* 2008; Cardoso *et al,* 2012). The use of cut-offs more adapted to the interference of necroinflammatory activity (expressed by higher ALT levels) on TE measurement, instead of fixed values, did not improve TE performances for estimating liver fibrosis. However, it needs to be validated in further studies, in different populations (Myers *et al,* 2010; Cardoso *et al,* 2012).

In the present study, variables significantly positively associated with the FibroScan values were AST, ALT, total serum bilirubin, prothrombin time and Child-Pugh grades as well as biochemical fibrosis markers including Fibrotest, Actitest, APRI, Forns index, and hyaluronic acid. The variables significantly negatively associated with the FibroScan values were platelets count and serum albumin level. These results were in agreement with those obtained by several previous studies on patients with CLDs of various causes including those with CHC, both in children (De Lédinghen *et al,* 2007; Fitzpatrick *et al,* 2013) and adults (Foucher *et al*, 2006, Coco *et al,* 2007; Ogawa *et al,* 2007, Zakareya *et al,* 2010). Those authors found that LSM was significantly correlated to the highest number of parameters, including total serum bilirubin, prothrombin time, INR, alkaline phosphatase, ϒ-glutamyl transferase (ϒ-GGT), AST, ALT, APRI, the Child-Pugh grade (significant positive), platelets count, serum albumin (significant negative correlations) with slight differences between different studies. The minor

differences between different studies could be due to different etiologies and number of patients of CLDs as well as differences in the severity of CLDs in the studied cohorts which had an influence on the results. These results indicate that LSM are related to the biological parameters and the major variations of the biochemical activity of CLDs suggesting that LSM by FibroScan reflects activity and progression of the chronic liver disease. Thus, in clinical practice, each LSM has to be interpreted taking into account the concurrent biochemical profile of the patient (Coco *et al,* 2007). Furthermore, Breton *et al.* (2009) reported that LSM was related to the occurrence of complications of cirrhosis where they found a significant relationship between FibroScan results and the presence of esophageal varices that indicates presence of portal hypertension syndrome in children with CLDs of varying etiology.

In this study, it was demonstrated that the predictive performance of TE showed progressive elevation with increasing stage of fibrosis with the highest performance in cirrhosis followed by advanced fibrosis, then significant fibrosis with AUROCs of 1.00, 0.815 and 0.652, respectively. These results coincided with that previously shown in several prior studies on patients with CLDs of various causes including those with CHC, both in children (De Lédinghen *et al,* 2007; Fitzpatrick *et al,* 2013) and adults (Castèra *et al,* 2005; Ziol *et al,* 2005; Coco *et al,* 2007; Ogawa *et al,* 2007; Sporea *et al,* 2008; Friedrich-Rust *et al,* 2008; Wit-

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ters *et al,* 2009). These authors found that TE values exhibited an excellent performance for the diagnosis of advanced fibrosis and cirrhosis. Controversy in these studies was observed only for the diagnostic performance in predicting significant fibrosis which varied from good to weak. It is possible that this variation is due to the difference in the severity of CLDs and sample size with relatively small numbers in each of the diagnostic groups (Friedrich-Rust *et al,* 2008). In this study as well as in most of the previously mentioned studies, TE demonstrated high performance in the detection of advanced fibrosis and cirrhosis; while TE performance is less reliable for the detection of early fibrosis stages (F1 &F2) as compared to more advanced stages of liver fibrosis. Furthermore, it has been shown that TE exhibits a similar performance for predicting significant fibrosis and higher accuracy to identify cirrhosis, as compared to other non-invasive tests in several previous studies in which the performances of TE and of several serum non-invasive biomarkers of liver fibrosis for predicting the presence of cirrhosis were compared in patients with CLDs of various causes including those with CHC (Coco *et al*, 2007; De Lédinghen *et al,* 2007; Friedrich-Rust *et al,* 2008; Castèra *et al,* 2009).

In the present work, the NPVs to exclude F3 or greater disease and cirrhosis were high (100%), whereas PPVs were modest (60.0%, 83.3%), at cut off values of 9.5 and 12.5kPa, respectively. These results coincided with that reported by Breton *et al.*

(2009) in children with CLDs. In addition, these results coincided with Castéra *et al.* (2009) who reported that the highly validated TE cut-off value of 12.5 kPa exhibited a PPV of 85% to predict the presence of cirrhosis and a NPV of 95% to exclude cirrhosis in adult patients with CHC. Likewise, Lupsor *et al.* (2008) and Myers *et al.* (2010) reported comparable results in adult patients with CHC. These results indicate that the main value of LSM is to exclude advanced fibrosis and cirrhosis as a screening test. However, TE cannot replace liver biopsy for the diagnosis of mild and moderate fibrosis stages (Myers *et al,* 2010). In contrast, Coco *et al.* (2007) found modest NPVs (78.8% and 81.6%), and high PPVs (93.8% & 97.8%), at cutoff values of 8.3 and 14 kPa, respectively.

It was reported that the adoption of TE as a screening test could potentially spare two thirds of CLDs patients from liver biopsies. Moreover, it was stated that a scoring system combining non-invasive markers including TE with some clinical features and biochemical serum markers may permit reliable differentiation of patients with advanced fibrosis vs. those with mild fibrosis among CLDs patients, and hence, easier selection of patients with a higher probability of significant pathology on liver biopsy (Yoneda *et al,* 2008). Because the prevalence of CLDs is high in many countries, this approach would be cost saving (Wong *et al,* 2010). However, Nobili *et al.* (2008) reported that it is unlikely for TE to show a good performance for screening purposes in unselected pediatric popula-

tions seen in primary care settings unlike those highly selected at a specialized tertiary care referral center. In contrast, Wong *et al.* (2010) reported that patients recruited at referral centers likely had more advanced disease and the NPVs to exclude advanced fibrosis and cirrhosis would be even higher in the primary care setting.

The limitations of the present study must be recognized. First, liver biopsy was used as the gold standard. In addition to sampling error, variability in histological interpretation may have contributed to an imperfect gold standard bias (Mehta *et al,* 2009). Nowadays, liver biopsy is the only reference standard, and biopsy specimens were assessed by an expert pathologist. Second, only a small number of patients with different fibrosis stages were included; additional large scale studies are necessary. Third, patients recruited at referral centers likely had more advanced disease. However, the NPVs to exclude advanced fibrosis and cirrhosis would be even higher in the primary care setting (Wong *et al,* 2010). Forth, obese subjects were not analyzed as LSM is impossible to obtain in this group. The problem was solved with the development of probes for obese subjects (De Lédinghen *et al,* 2009). In addition, patients having ascites were not analyzed as LSM is impossible to obtain in this group as the elastic shear waves do not propagate through liquids. However, the presence of ascites in patients with CLDs indicates the presence of cirrhosis. Fifth, the relatively small number of subjects recruited, reflecting the difficulty in obtaining

liver biopsy samples and an accurate clinical framing even in a specialized pediatric referral center, may not allow reaching definitive conclusions as regard the optimal cut-off values (Nobili *et al,* 2008). Also, it is expensive and has some difficulties in children less than 2 years as a result of their irritability and cannot determine the underlying cause of CLDs and only it can follow its progression (Goldschmidt *et al,* 2013).

Conclusion

Measurement of liver stiffness using FibroScan is an accurate and clinically useful technique for the noninvasive prediction of the severity of hepatic fibrosis among children with CHC. Its use for the follow up and management of those patients could be of great interest and should be evaluated further.

Acknowledgements

References

Arena U, Vizzutti F, Abraldes JG, *et al*., 2008: Reliability of transient elastography for the diagnosis of advanced fibrosis in chronic hepatitis C. Gut 57:1288-93.

Bedossa, P, Dargere, D, Paradis, V, 2003: Sampling variability of liver fibrosis in chronic hepatitis C. Hepatology 38:1449-57

Bedossa, P, Poynard, T, 1996: An algorithm for the grading of activity in

chronic hepatitis C: The METAVIR cooperative study group. Hepatology 24:289-93.

Benvegnu, L, Gios, M, Boccato, S, Alberti, A, 2004: Natural history of compensated viral cirrhosis: a prospective study on the incidence and hierarchy of major complications. Gut 53: 744-9.

Breton, E, Bridoux-Henno, L, *et al.*, 2009: Value of transient elastography in noninvasive assessment in children's hepatic fibrosis. Arch. Pediatr. 16: 1005-10

Cardoso, AC, Carvalho-Filho, RJ, Stern, C, Dipumpo, A, *et al*, 2012: Direct comparison of diagnostic performance of transient elastography in patients with chronic hepatitis B and chronic hepatitis C. Liver Int. 32, 4: 612-21.

Castèra, L, Le Bail, B, Roudot, F, *et al*. 2009: Early detection in routine clinical practice of cirrhosis and esophageal varices in chronic hepatitis C: comparison of transient elastography (FibroScan) with standard laboratory tests and non-invasive scores. J. Hepatol. 50:59-68.

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

Coco, B, Oliveri, F, Maina, AM, Ciccorossi, P, *et al*. 2007: Transient elastography: a new surrogate marker of liver fibrosis influenced by major changes of transaminases. J. Viral Hepatol. 14:360-9.

Colloredo, G, Guido, M, Sonzogni, A, *et al*, 2003: Impact of liver biopsy size on histological evaluation of chronic viral hepatitis: the smaller the sample, the milder the disease. J. Hepatol. 39:239-44.

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

De Lédinghen V, Fournier, C, Foucher, J, *et al*, 2009: New FibroScan probe for obese patients: A pilot study of feasibility and performances in patients with BMI >30 kg/m^2. J. Hepatol. 50, 1:S359.

Fitzpatrick, E, Quaglia, A, Vimalesvaran, S, *et al*, 2013: Transient elastography is a useful noninvasive tool for the evaluation of fibrosis in pediatric chronic liver disease. J. Pediatr. Gastroenterol. Nutr. 56, 1:72-6.

Fontana, RJ, Lok, AS, 2002: Noninvasive monitoring of patients with chronic hepatitis C. Hepatol. 36:S57-64.

Forns, X, Ampurdanes, S, Llovet, J M, *et al*, 2002: Identification of chronic hepatitis C patients without hepatic fibrosis by a simple predictive model. Hepatol. 36: 986-92.

Foucher, J, Chanteloup, E, Vergniol, J, *et al*, 2006: Diagnosis of cirrhosis by tra-nsient elastography (FibroScan): a prospective study. Gut 55:403-8.

Fraquelli, M, Rigamonti, C, Casazza, G, *et al.*, 2011: Etiology-related determinants of liver stiffness values in chr-

onic viral hepatitis B or C. J. Hepatol. 54:621-8.

Friedrich-Rust, M, Ong, MF, Martens, S, *et al*, **2008:** Performance of transient elastography for the staging of liver fibrosis: a meta-analysis. Gastroenterol. 134: 960-74.

Fung, YC, 1993: Biomechanics: Biomechanical Properties of Living Tissues. 2nd edition. New York, Springer Verlag**.**

Goldschmidt, I, Streckenbach, C, Dingemann, C, *et al.*, **2013:** Application and limitations of transient liver elastography in children. J. Pediatr. Gastroenterol. Nutr. 57, 1:109-13.

Imbert-Bismut, F, Ratziu, V, Pieroni, L, *et al*, **2001:** Biochemical markers of liver fibrosis in patients with hepatitis C virus infection: a prospective study. Lancet 357:1069-75.

Janssens, F, Spahr, L, Rubbia-Brandt, L, *et al*, **2010:** Hepatic amyloidosis increases liver stiffness measured by transient elastography. Acta Gastroenterol. Belg. 73:52-4**.**

Khan, JA, Khan, FA, Dilawar, M, *et al*, **2007:** Serum hyaluronic acid as a marker of hepatic fibrosis. JCPSP 17: 323-6.

Lachaux, A, Le Gall, C, Chambon, M, *et al*, **1995:** Complications of percutan-eous liver biopsy in infants and children. Eur. J. Pediatr. 154:621-3.

Lupşor M, Badea R, Stefănescu H, Grigorescu M, *et al.,* **2008**: Analysis of his-topathological changes that influence liver stiffness in chronic hepatitis C. results from a cohort of 324 patients. J. Gastrointest. Liver Dis. 17, 2:155-63.

Mederacke, I, Wursthorn, K, Kirschner, J, *et al*, **2009:** Food intake increases liver stiffness in patients with chronic or resolved hepatitis C virus infection. Liver Int. 29:1500-6.

Mehta, SH, Lau, B, Afdhal, NH, Thomas, DL, 2009: Exceeding the limits of liver histology markers. J. Hepatol. 50:36-41.

Millonig, G, Friedrich, S, Adolf, S, Fonouni, H, *et al*, **2010:** Liver stiffness is directly influenced by central venous pressure. J. Hepatol. 52: 206-10.

Millonig, G, Reimann, FM, Friedrich, S, *et al*, **2008:** Extrahepatic cholestasis increases liver stiffness (Fibro Scan) irrespective of fibrosis. Hepatol. 48:1718-23.

Myers, RP, Elkashab, M, Crotty, P, *et al*, **2010:** Transient elastography for the noninvasive assessment of liver fibrosis: A multicentre Canadian study. Can. J. Gastroenterol. 24, 11:661-70.

Nobili, V, Vizzutti, F, Arena, U, *et al*, **2008:** Accuracy and reproducibility of transient elastography for the diagnosis of fibrosis in pediatric nonalcoholic steatohepatitis. Hepatol. 48:442-8**.**

Ogawa, E, Furusyo, N, Toyoda, K, Takeoka, H, *et al*, **2007:** Transient elastography for patients with chronic hepatitis B and C virus infection: Noninvasive, quantitative assessment of liver fibrosis. Hepatol. Res. 37:1002-10.

Regev, A, Berho, M, Jeffers, LJ, *et al*, **2002:** Sampling error and intraobserver variation in liver biopsy in patients with chronic HCV infection. Am. J. Gastro-enterol. 97, 10:2614-8.

Sagir, A, Erhardt, A, Schmitt, M, Häussinger, D, 2008: Transient elastography is unreliable for detection of cirrhosis in patients with acute liver damage. Hepatol. 47:592-5.

Saito, H, Tada, S, Nakamoto, N, *et al*, 2004: Efficacy of non-invasive elastome- try on staging of hepatic fibrosis. Hepatol. Res. 29: 97-103.

Sandrin, L, Fourquet, B, Hasque-noph, JM, *et al*, 2003: Transient elastography: a new noninvasive method for assessment of hepatic fibrosis. Ultrasound Med. Biol. 29:1705-13

Scheimann, AO, Barrios, JM, Al-Tawil, YS, *et al*, 2000: Percutaneous liver bio-psy in children: impact of ultrasonography and spring-loaded biopsy needles. J. Pediatr. Gastroenterol. Nutr. 31:536-9.

Seeff, LB, 2002: Natural history of chronic hepatitis C. Hepatol.36:S35-46.

Sporea, I, Şirli, R, Deleanu, A, Tudora, A, *et al*, 2008: Comparison of the liver stiffness measurement by transient elastography with liver biopsy. Wld. J. Gastroenterol. 14, 42:6513-7

Talwalkar, JA, Kurtz, DM, Schoenleber, SJ, *et al*, 2007: Ultrasound-based transient elastography for the detection of hepatic fibrosis: systematic review and metaanalysis. Clin. Gastroenterol. Hepatol. 5:1214-20

The French METAVIR Cooperative Study Group, 1994: Intraobserver and interobserver variations in liver biopsy interpretation in patients with chronic hepatitis C. Hepatol. 20:15-20.

Wai, CT, Greenson, JK, Fontana, RJ, *et al*, 2003: A simple noninvasive index can predict both significant fibrosis and cirrhosis in patients with chronic hepatitis C. Hepatol. 38:518-26.

Witters, P, De Boeck, K, Dupont, L, *et al*, 2009: Non-invasive liver elastography (FibroScan) for detection of cystic fibrosis-associated liver disease. J. Cyst Fibros. 8:392-9.

Wong, VW, Vergniol, J, Wong, GL, Foucher, J, *et al.*, 2010: Diagnosis of fib-rosis and cirrhosis using liver stiffness measurement in nonalcoholic fatty liver disease. Hepatol. 51:454-62.

Yoneda, M, Mawatari, H, Fujita. K, *et al*, 2008: Noninvasive assessment of liver fibrosis by measurement of stiffness in patients with nonalcoholic fatty liver disease (NAFLD). Dig. Liver Dis. 40:371-8.

Zakareya, T, Abdel Razek, W, Allam, N, *et al*, 2010: The utility of transient elastography in predicting complications of chronic liver disease. Nile Liver J. 1, 1:49-57.

Ziol, M, Handra-Luca, A, Kettaneh, A *et al*, 2005: Noninvasive assessment of liver fibrosis by measurement of stiffness in patients with chronic hepatitis C. Hepatol. 41:48-54.

Explanation of figures

Fig. 1: Box plot of LSM, histopathological fibrosis stages (A) and necroinflammatory activity grades (B) among patients with CHC.

Fig. 2: Correlation between LSM and histopathological fibrosis stages (A) and necroinflammatory activity grades (B).

Fig. 3: ROC curves of LSM of enrolled patients as a diagnostic tool for prediction of mild liver fibrosis (A $F \geq 1$), significant liver fibrosis (B $F \geq 2$), advanced liver fibrosis (C $F \geq 3$) and liver cirrhosis (D $F = 4$).

Fig. 1 A Histopathology fibrosis score

Fig. 1 B Histopathology activity grade score

Fig. 2 A Histopathological fibrosis score

Fig. 2 B Histopathological necroinflammatory activity grade

ROC Curve

AUROC=0.031

Fig. 3 A Sensitivity

ROC Curve

AUROC=0.652

Fig. 3 B Sensitivity

ROC Curve

AUROC=0.815

Fig. 3 C Sensitivity

ROC Curve

AUROC=1.00

Fig. 3 D Sensitivity