## Abstract

**Aim:** Liver biopsy is the gold standard for assessing liver fibrosis (LF) after liver transplantation (LT), but its invasiveness limits its utility. This study aimed to evaluate the usefulness of liver stiffness measurement (LSM) via acoustic radiation force impulse (ARFI) imaging to assess LF after LT.

**Methods:** Between September 2013 and January 2017, 278 patients who underwent liver biopsy after LT in Kyoto University Hospital were prospectively enrolled. Liver stiffness measurement was performed via ARFI imaging; its value was expressed as shear wave velocity (Vs) [m/s]. The LF was evaluated according to the METAVIR scores (F0–F4). The diagnostic performance of Vs for F2≤ and F3≤ was assessed and compared with that of laboratory tests using receiver operating characteristic (ROC) analysis.

**Results:** The median Vs values increased according to the progression of LF (F0: 1.18±0.27, F1: 1.35±0.42, F2: 1.55±0.54, F3: 1.84±0.50). The Vs had the highest area under the ROC curve (AUROC) for the prediction of both F2≤ and F3≤ fibrosis (F2: 0.77 and F3: 0.85). With the cut-off value of Vs >1.31, sensitivity, specificity, positive predictive value, and negative predictive value were 89.4%, 53.3%, 37.3%, and 94.2% in predicting F2≤, respectively. Vs diagnosed LF better than any laboratory tests regardless of the type of primary disease.

**Conclusions:** ARFI helps assess graft LF after LT. The high sensitivity suggested that ARFI may reduce the frequency of liver biopsies by detecting patients who are unlikely to have significant fibrosis after liver transplantation. (Unique trial number: UMIN R000028296)

**Keywords**

Acoustic radiation force impulse, biopsy, fibrosis, liver stiffness measurement, liver transplantation, ultrasound-based elastography

**Abbreviations:**

ALB, albumin

APRI, Aspartate transaminase to Platelet Ratio Index

ARFI, acoustic radiation force impulse

AUROC, area under the receiver operating characteristic curve

BMI, body mass index

CI, confidence interval

FIB-4, Fibrosis-4

HCV, hepatitis C virus

LT, liver transplantation

LF, liver fibrosis

LSM, liver stiffness measurement

OR, odds ratio

PT-INR, prothrombin time (international normalized ratio)

ROC, receiver operating characteristic

Vs, shear wave velocity

T-Bil, total bilirubin

TP, total protein

## Text

### Introduction

Liver transplantation (LT) has become the treatment of choice for end-stage liver diseases worldwide.[1] Although short- and long-term results have been improved with the development of operative techniques and perioperative management,[2] they still leave room for further improvement.[3] Graft fibrosis is a serious condition after LT, which can cause graft dysfunction leading to graft loss and retransplantation. Early recognition and treatment intervention are of paramount importance in improving long-term prognosis of LT patients.

Percutaneous liver biopsy has been the gold standard for the assessment of graft condition including acute cellular rejection and graft fibrosis in the post-transplant setting.[4] Histopathological examination of biopsy specimens can reveal not only rejection and fibrosis of graft liver but also the recurrence of primary diseases such as viral hepatitis, primary biliary cirrhosis, and primary sclerosing cholangitis.[5-8] However, liver biopsy is associated with poor compliance owing to its invasiveness and possible complications. Less invasive methods are strongly desired especially for long-term follow-up.[9]

Recently, liver stiffness measurement (LSM) has been developed as a noninvasive method for assessing liver fibrosis (LF).[10, 11] Previous studies reported that LSM via

magnetic resonance elastography and ultrasound-based elastography methods such as acoustic radiation force impulse (ARFI) and FibroScan® (Echosens, Paris, France) were useful for evaluating LF.[12-20] In addition, the usefulness of LSM in predicting development of clinical manifestations of liver cirrhosis such as esophageal varices, portal hypertension, ascites, and hepatocellular carcinoma has been reported in several studies.[21, 22] Our previous report showed that LSM via ARFI was useful for predicting post-hepatectomy liver failure in patients with hepatocellular carcinoma.[23] The utility of FibroScan® for the assessment of graft fibrosis after LT has been reported by several studies,[24-27] however, the utility of ARFI has been only evaluated by a few studies involving small number of patients with inconclusive results.[28-32]

This study is the largest post LT cohort study for assessment of LSM using ARFI imaging. The aim of this study was to evaluate the usefulness of LSM via ARFI imaging for the evaluation of graft conditions such as fibrosis.

## Methods

### PATIENTS

We prospectively collected and analyzed the clinicopathological data of 278 patients who underwent liver biopsies for post-transplant assessment of graft liver at Kyoto University

Hospital between September 2013 and January 2017. Written informed consent was obtained from all patients after liver biopsy.

The study protocol was approved by the Ethics Committee of the Kyoto University Graduate School and Faculty of Medicine (approval code: E1992) and was registered with the University Hospital Medical Information Network (unique trial number: UMIN R000028296).

## DATA COLLECTION

The clinicopathological data including sex, age, body mass index (BMI), primary disease, splenectomy on LT, blood compatibility, reason for biopsy, and interval between biopsy and LT were collected.

All patients underwent blood examinations, including liver function tests (measurement of platelet count, international normalized ratio of prothrombin time (PT-INR), alanine transaminase level, ɤ-glutamyl transpeptidase level, total bilirubin (T-Bil) level, total bile acid level, total protein (TP) level, and albumin (ALB)) level and the levels of fibrosis markers such as hyaluronic acid and type 4 collagen), on the same day of liver biopsy. Aspartate transaminase to Platelet Ratio Index (APRI) and Fibrosis-4 (FIB-4) index were calculated according to the equations previously reported.[33-35]

## LIVER BIOPSY AND HISTOLOGICAL ASSESSMENT

Liver biopsy examination was performed when clinically indicated (e.g., when rejection was suspected) or at designated intervals (so-called protocol biopsy), with informed consent. An 18-G biopsy needle was routinely used, and the minimal acceptable size of liver biopsy specimens was 15 mm. Biopsy specimens were fixed in formalin, embedded in paraffin, and stained with hematoxylin–eosin and Masson's trichrome. Necroinflammatory activity (A0–A3) and fibrosis stage (F0–F4) were assessed according to METAVIR scores.[36]

## LIVER STIFFNESS MEASUREMENT

The liver stiffness was evaluated using ACUSON S2000 (Mochida Siemens Medical Systems, Tokyo, Japan) as previously reported.[37] In the ARFI, the value of tissue stiffness in a region of interest is expressed as the shear wave velocity (Vs) in meters per second (m/s). The LSM was performed in the same day of the biopsy. The examined patients were laid in the supine position. The region of interest (fixed-dimension 1 × 0.5-cm box) was chosen in the lobe from the intercostal space or epigastric scan at a depth of 4–6 cm from the surface and free of large vascular structures. Vs values were measured 10 times

in every patient, and the mean value in m/s was calculated. Valid ARFI measurements were obtained in all patients. The operators were blinded to the clinical data.

## STATISTICAL ANALYSIS

All statistical analyses were performed using SAS software (JMP 12.0.; SAS Institute Inc.). Continuous variables were expressed as mean values ± standard deviation or medians with ranges and compared using Student's t-test, the Kruskal–Wallis test, or the Wilcoxon rank-sum test. Categorical variables were compared using the chi-square test or Fisher's exact test as appropriate. The diagnostic significance of Vs and laboratory tests for F2≤ were evaluated in multivariable analysis. The variables included in the analysis were selected based on the results of univariate analysis ($P$<0.05). The diagnostic performance for fibrosis score F2≤ and F3≤ was assessed using receiver operating characteristic (ROC) analysis, and the area under the ROC curve (AUROC) was calculated. The optimal cut-off values were determined to maximize the sum of sensitivity and specificity. ROC curves were compared using DeLong test. The interaction between the variables was tested using Spearman's rank correlation coefficient. A $P$-value of <0.05 was considered statistically significant.

## Results

### PATIENTS' CHARACTERISTICS

Table 1 shows the patients' characteristics. The study population consisted of 139 men and 139 women with a median age of 48.0 ± 18.3 years and median BMI of 22.3±3.8. The primary liver diseases were biliary atresia (n=83, 30.0%), hepatitis C virus (HCV) cirrhosis (n=70, 25.2%), primary biliary cirrhosis (n= 38, 13.7%), fulminant hepatitis (n=18, 6.5%), hepatitis B virus cirrhosis (n=16, 5.8%), primary sclerosing cholangitis (n=12, 4.3%), alcohol (n=6, 2.2%), and others (n=35, 12.6%). The types of grafts were whole liver, right lobe, left lobe, and lateral segment in 19 (6.8%), 134 (48.2%), 63 (22.7%), and 62 patients (22.3%), respectively. Splenectomy was performed in 89 patients (32.0%). The median time between LT and liver biopsy was 8.3 ± 6.8 years.

### DIAGNOSTIC PERFORMANCE OF ARFI FOR PATHOLOGIC LIVER FIBROSIS

The METAVIR fibrosis score was F0 in 74 patients (26.6%), F1 in 138 (49.6%), F2 in 52 (18.7%), F3 in 14 (5.0%), and F4 in no patient, whereas the METAVIR activity score was A0 in 111 patients (39.9%), A1 in 148 (53.2%), A2 in 18 (6.5%), and A3 in 1 (0.4%).

The median Vs values increased according to the progression of LF: 1.18 (0.78-1.92) m/s in F0, 1.35 (0.72-3.54) m/s in F1, 1.55 (1.05-3.37) m/s in F2, and 1.84 (1.41-2.97) m/s in

F3 (Figure 1). The median Vs appeared to be weakly correlated with METAVIR activity score in A0–A2, but not in A3 presumably because of insufficient sample number. The Vs was not correlated with rejection activity index score.[38] The diagnostic performance of Vs and laboratory tests for LF stage was analyzed (Table 2). The Vs had higher AUROC than any laboratory tests for the prediction of both F2≤ and F3≤ fibrosis (F2≤: AUROC 0.77, 95% confidence interval [CI] 0.70–0.82; F3≦: AUROC 0.85, 95% CI 0.77–0.91). The cut-off values for F2≦ and F3≤ were, respectively, 1.31 m/s and 1.53 m/s, and the sensitivity, specificity, positive predictive value, negative predictive value, positive likelihood ratio, and negative likelihood ratio for F2≤ were 89.4%, 53.3%, 37.3%, 94.2%, 1.91, and 0.20, respectively, and those for F3≤ were 92.9%, 69.7%, 14.0%, 99.5%, 3.07, and 0.10, respectively (Table 3).

## A MODEL FOR PREDICTION OF F2≤ FIBROSIS

The univariable logistic regression analysis of each variable and the multivariable stepwise selection in predicting F2≤ were performed (Table 4). Significant differences were observed in Vs ($p<0.001$), PT-INR ($P=0.016$), T-Bil level ($P=0.009$), total bile acid level ($P<0.001$), TP level ($P=0.004$), ALB level ($P=0.027$), hyaluronic acid level ($P=0.037$), and type 4 collagen level ($P=0.006$) between the patients with and without

F2≤ in univariable analysis. Vs (odds ratio [OR]: 2.43, 95% CI: 1.63–3.79, *P*<0.001), PT-INR (OR: 1.93, 95%CI: 1.20–3.19, *P*=0.006), and TP level (OR: 1.66, 95%CI: 1.11–2.56, *P*=0.013) remained significant in multivariable analysis.

The F2≤ risk index incorporating these three parameters was generated as follows:

[F2≤ risk index] = 1.74 × Vs + 1.74 × PT-INR + 0.56 × TP

The AUROC of the F2≤ risk index was 0.79 (95% CI 0.72–0.84). However, the predictive power of this risk index was not significantly better than that of Vs alone (*P*=0.359) (Figure 2).

## COMPARISON OF DIAGNOSTIC ACCURACY FOR F2≤ IN VARIOUS SUBPOPULATIONS

The serum markers can be affected by various factors such as primary disease and presence of rejection, which may have resulted in inferior diagnostic performance of laboratory tests in our heterogeneous study population. The diagnostic performance for F2≤ was compared between Vs and laboratory tests in various subpopulations, which are expected to be more homogeneous than the entire cohort. In the subpopulation where the primary disease was biliary atresia, diagnostic power of laboratory tests was scarcely affected compared with the whole cohort, leaving statistically significant difference from

that of Vs in most of the serum markers (Table 5). In the HCV cohort, on the other hand, several serum markers including PT-INR, T-Bil, TP, ALB, and hyaluronic acid yielded improved diagnostic performance compared with the entire cohort, although Vs still showed higher AUROC value than any serum parameters. The same trend was observed also in the splenectomized cohort (Table 6).

## IMPACT OF SCANNING SITE ON DIAGNOSTIC PERFORMANCE OF Vs

We analyzed the variation (Vs standard deviation/Vs mean) of Vs to evaluate the reproducibility of ARFI examination. The variation of epigastric scan (n=71) was significantly larger than that of intercostal scan (n=207) in the evaluation of liver stiffness ($P$<0.001, 0.11±0.005, 0.08±0.003, respectively), indicating better reproducibility of LSM in intercostal scan than in epigastric scan. As a result, the AUROC of Vs in intercostal scan was significantly better than that in epigastric scan for prediction of F2≤ ($P$=0.046, AUROC: 0.80, 95% CI 0.72–0.86; AUROC 0.63, 95% CI 0.47–0.77, respectively) (Figure 3). Reflecting this, Vs predicted F2≤ more accurately in the right lobe graft than in the lateral segment and left lobe graft *(P*=0.04, AUROC: 0.83, 95% CI: 0.70–0.91; AUROC: 0.67, 95% CI: 0.59–0.77).

## Discussion

Currently, liver biopsy is the gold standard for the evaluation of LF. However, major complications were reported to occur in 0.6%, and mortality rate was 0.09% in a systematic review.[9] Additionally, Bedossa et al. reported that diagnostic accuracy was only 65% with biopsy specimens with 15-mm length and 75% even with 25-mm length, indicating suboptimal accuracy of needle biopsy due to sampling variability.[39] The demand for less invasive and more accurate diagnostic tool for assessing LF is continually growing.

The usefulness of serum markers or composite markers as noninvasive methods for the evaluation of LF has been reported. Benlloch et al. and Cross et al. reported the usefulness of the original formula of predicting fibrosis using serum markers and time from LT.[40, 41] Toniutto et al. and Kitajima et al. reported the utility of APRI or FIB-4 index in HCV patients after LT.[42, 43] Additionally, Carrion et al. reported that direct fibrosis markers such as tissue inhibitor of metalloproteinase-1, amino-terminal propeptide of type III procollagen, and hyaluronic acid were better than APRI or Benlloch's original formula in predicting fibrosis in HCV patients after LT.[44] However, these studies indicated that the diagnostic performance of serum markers were not reproducible in the studies other than the original one and questioned the validity in the external cohort. Previous studies

reported that FibroScan® was useful for evaluation of LF,[13-15] and a meta-analysis has shown that its efficacy for evaluation of LF was equivalent to composite markers in non-transplant patients.[45] Recently, several studies reported that the usefulness of ARFI as a device to evaluate LF [16-18] and a meta-analysis showed ARFI is as useful as FibroScan® in non-transplant patients.[46] On the other hand, in liver transplant patients, several studies reported that FibroScan® is more useful than serum or composite markers in evaluation of LF.[24-27] Accordingly, ARFI is expected to be better than serum markers and composite markers for the evaluation of fibrosis in liver transplant patients. However, only few small studies have been conducted that assessed the efficacy of ARFI in the post-transplant setting, and these results are inconclusive, probably because of a small sample size.[28-32] Additionally, measurement using Fibroscan, which is based on the M-mode and A mode imaging of ultrasonography, is not a realtime procedure. Its potential limitation is that measurement is difficult in obese patients or patients with ascites, and it is affected by the operator's experience.[47] In contrast, ARFI technology, which is a simple real-time procedure based on B-mode imaging, makes it possible to observe the ROI, adapt measurement depth according to the skin to liver capsule distance, and measure slim and obese patients as well as patients with ascites.[48] A previous study reported that ARFI is better than FibroScan® for patients with ascites, higher BMI, and longer abdominal

perimeter.[49] Therefore, ARFI might be more useful than FibroScan®, and we examined the usefulness of ARFI in evaluating fibrosis of a transplanted liver.

We confirmed that Vs, the liver stiffness value measured via ARFI, was significantly correlated with the stage of LF. Vs was better than any serum or composite markers for the diagnosis of significant (F2≤) and advanced (F3≤) fibrosis. When the cut-off values were 1.31 and 1.53, Vs had good negative predictive value and negative likelihood ratio in predicting F2≤ and F3≤, respectively, indicating that LSM via ARFI is useful for identifying patients who are unlikely to have significant fibrosis and can effectively rule out such patients to reduce the frequency of nonessential liver biopsies.

A multivariable analysis was further performed, and a model for predicting significant fibrosis (F2≤) was built. Selection of TP, not ALB, presumably reflects increasing globulin fraction in fibrotic patients[50, 51] and is concordant with the Benlloch's study where ALB/TP ratio was identified as a predictive factor for significant fibrosis.[40] The obtained equation of this model, the Vs-PT-INR-TP index, had good diagnostic accuracy for F2≤ fibrosis (AUROC 0.79). However, even Vs alone had an AUROC of 0.77, without significant difference from that of Vs-PT-INR-TP index, suggesting that addition of laboratory tests to Vs adds little diagnostic information regarding LF in post-transplant setting.

In non-transplant settings, several biomarkers and composite markers have been shown to be useful for the prediction of LF.[33-35] The present study showed that the usefulness of laboratory tests for diagnosis of graft fibrosis after LT was limited, as demonstrated by low AUROC values of these markers. The reason for the discrepant efficacy of laboratory tests for the estimation of LF between non-transplant patients and post-transplant patients is a matter of interest. In transplant recipients, laboratory tests can be influenced by various factors other than the progression of fibrosis. These include rejection, recurrence of primary disease, biliary complications, presence of the spleen, and immunosuppressants, and they might have affected diagnostic performance of blood tests. For example, rejection may increase aspartate transaminase level irrespective of LF and affect APRI, which incorporate aspartate transaminase into its formula. On the other hand, liver stiffness is hardly influenced by the conditions other than LF, as demonstrated by weaker correlation with activity score or rejection activity index score than with fibrosis score. Notably, the presence of the spleen may affect various laboratory tests such as measurement of platelet count and T-Bil, as demonstrated by previous studies showing splenectomy increased platelet count and T-Bil level.[52-55] This is in agreement with our finding that diagnostic performance of laboratory tests was improved in splenectomized patients. The present study emphasizes the usefulness of LSM for diagnosis of graft

fibrosis as Vs is consistently reliable in predicting graft fibrosis without being affected by patients' background.

Diagnosis of LF based on ARFI does have several limitations. First, LSM with epigastric scan was less reproducible than that with intercostal scan, resulting in less reliable diagnostic performance. A previous study reported that ARFI examination can be performed more accurately for the right lobe than that for the left lobe in evaluating fibrosis.[56] Toshima et al. similarly reported that the AUROC for diagnosing LF by the Vs of intercostal scan for the right lobe was significantly higher than for that of epigastric scan for the left lobe, and the standard deviation of the measured values for the right lobe was significantly lower than that of the left lobe.[57] The reason for these differences was assumed that the epigastric scan for the left lobe surrounded by the diaphragm, stomach, and aorta may be influenced by respiratory fluctuations, the presence of food in the stomach, and the pulsation of the aorta. Hence, as the accuracy of ARFI examination may be influenced by not only the scanning site, but also the scanning lobe, we have to keep in mind that LSM by ARFI should be performed in intercostal scan if possible and that the diagnostic reliability is reduced when ARFI is performed in epigastric scan due to inadequate window for intercostal scan. Second, ARFI did not show good specificity and positive predictive value in evaluation for F2≤

or F3≤. Therefore, we have to be aware that false positives are frequently encountered in the evaluation of severe fibrosis via ARFI imaging.

In conclusion, LSM via ARFI imaging is very useful in predicting graft fibrosis after liver transplantation, regardless of the type of the primary disease or patient background and can minimize the frequency of nonessential liver biopsies by detecting patients who are unlikely to have significant fibrosis after liver transplantation.

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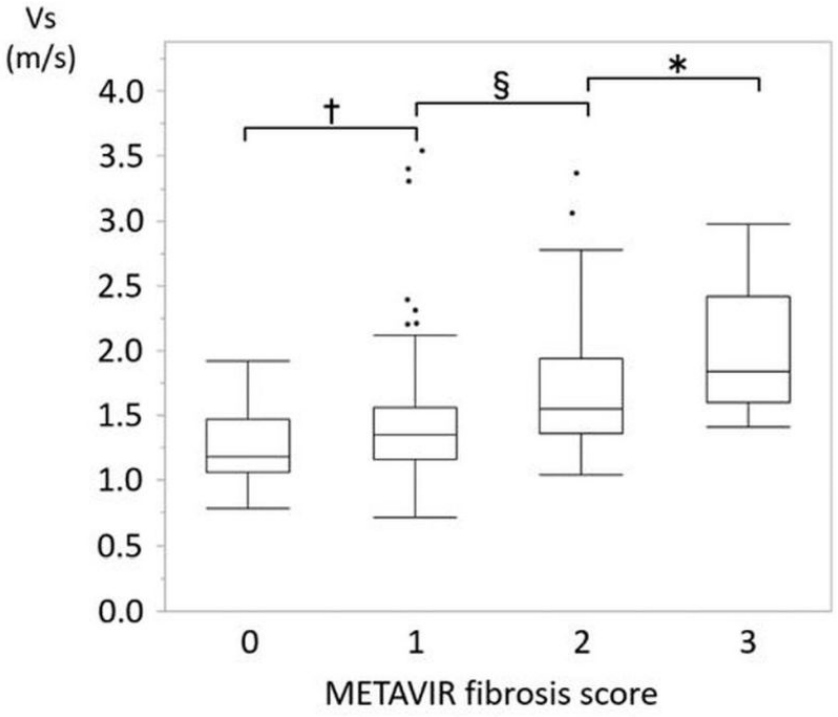


Figure 1. Correlation between shear wave velocity (Vs) and METAVIR fibrosis score

The differences between the abutting stages were evaluated using the Wilcoxon rank-sum test. Vs, shear wave velocity *: $p<0.05$, †: $p<0.01$, §: $p<0.0001$

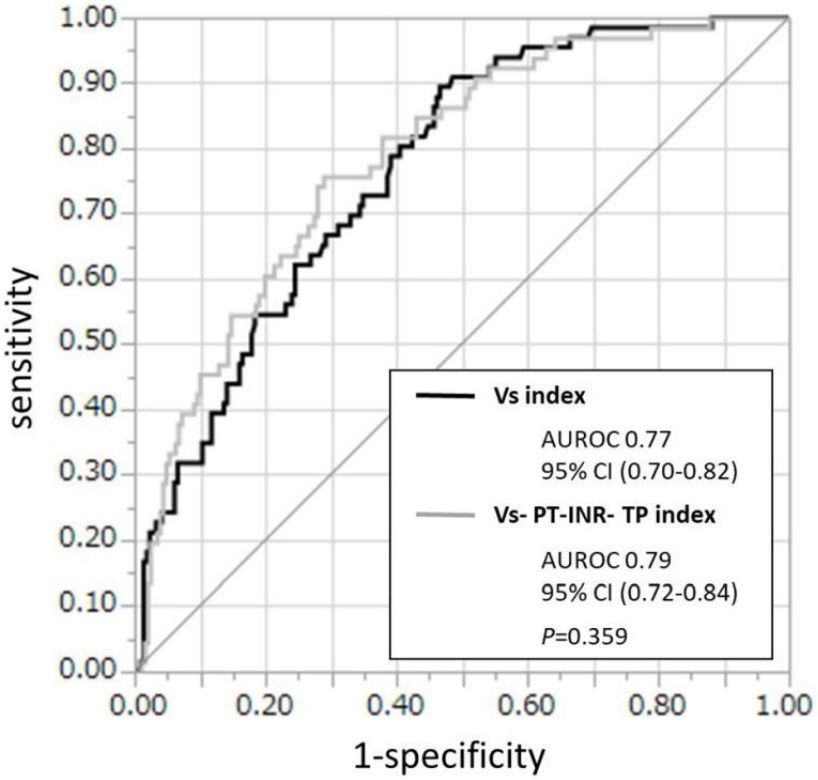


Figure 2. Comparison of ROC curve between the Vs-PT-INR-TP index and Vs for diagnosis of F2≤

AUROC, area under the receiver operating characteristic curve; CI, confidence interval; international normalized ratio of prothrombin time, PT-INR; TP, total protein; Vs, shear wave velocity

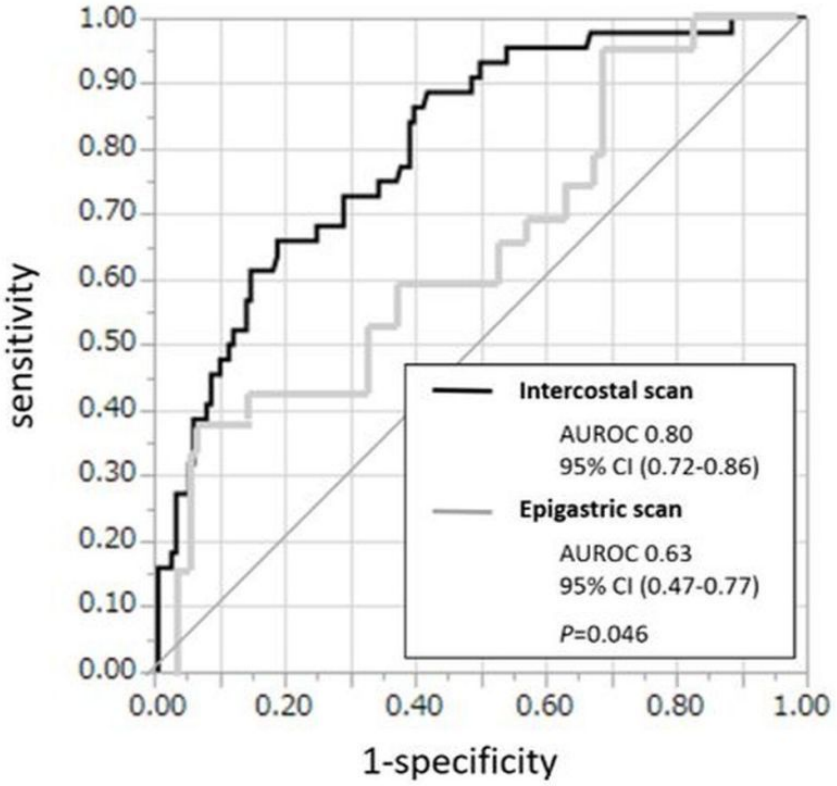


Figure 3. Comparison of diagnostic ability for F2≤ between intercostal scan and epigastric scan

AUROC, area under the receiver operating characteristic curve; CI, confidence interval

Table 1: Study population and patients' characteristics

| Characteristics | | |
|---|---|---|
| Male/female | | 139/139 (50%/50%) |
| Age (years) | | 48.0±18.3 |
| BMI | | 22.3±3.8 |
| METAVIR score | | |
| | A0/A1/A2/A3 | 111/148/18/1 (39.9%/53.2%/6.5%/0.4%) |
| | F0//F1/F2/F3/F4 | 74//138/52/14/0 (26.6%/49.6%/18.7%/5.0%/0%) |
| Primary disease | | |
| | Biliary atresia | 83 (30.0%) |
| | HCV-LC | 70 (25.2%) |
| | PBC | 38 (13.7%) |
| | Fulminant hepatitis | 18 (6.5%) |
| | HBV-LC | 16 (5.8%) |
| | PSC | 12 (4.3%) |
| | Alcohol | 6 (2.2%) |
| | Others | 35 (12.6%) |
| Type of graft | Whole/right/left/lateral | 19/ 134/ 63/ 62 (6.8%/ 48.2%/ 22.7%/ 22.3%) |
| Splenectomy (+/-) | | 89/189 (32.0% / 68.0%) |
| Blood compatibility | | |
| | Identical | 170 (61.2%) |
| | Compatible | 57 (20.5%) |
| | Incompatible | 39 (14.0%) |

| | | |
|---|---|---|
| | Unknown | 12 (4.3%) |
| Reason for biopsy | | |
| | Protocol biopsy | 199 (71.6%) |
| | Pre-antiviral therapy | 15 (5.0%) |
| | Biopsy on clinical manifestation | 64 (23%) |
| Postoperative period (years) | | 8.3±6.8 |

Continuous variables are expressed as mean value±standard divisions.

Categorical variables are expressed as number of patients.

BMI, body mass index; HCV, hepatitis C virus; LC, liver cirrhosis; LT, liver transplantation; PBC, primary biliary cirrhosis; PSC, primary sclerosing cholangitis

Table 2: Receiver operating characteristic analysis of fibrosis markers for predicting liver fibrosis

| | F2≤ | | F3≤ | |
|---|---|---|---|---|
| | AUROC | 95% CI | AUROC | 95% CI |
| Vs | 0.77 | 0.70–0.82 | 0.85 | 0.77–0.91 |
| Platelet count | 0.57§ | 0.49–0.65 | 0.50‡ | 0.33–0.67 |
| PT-INR | 0.63† | 0.55–0.71 | 0.73 | 0.58–0.84 |
| Alanine transaminase | 0.54§ | 0.46–0.62 | 0.61† | 0.42–0.77 |
| γ-Glutamyl transpeptidase | 0.47§ | 0.39–0.55 | 0.65* | 0.48–0.79 |
| Total bilirubin | 0.58‡ | 0.50–0.66 | 0.76 | 0.60–0.87 |
| Total bile acid | 0.56§ | 0.48–0.64 | 0.79 | 0.64–0.89 |
| Total protein | 0.60† | 0.52–0.68 | 0.59† | 0.41–0.75 |
| Albumin | 0.54§ | 0.45–0.62 | 0.70 | 0.49–0.85 |
| Hyaluronic acid | 0.56§ | 0.47–0.65 | 0.69 | 0.49–0.84 |
| Type 4 collagen | 0.64† | 0.55–0.72 | 0.72 | 0.55–0.85 |
| APRI | 0.62† | 0.55–0.69 | 0.65† | 0.49–0.79 |
| FIB-4 index | 0.52§ | 0.44–0.60 | 0.56‡ | 0.40–0.70 |

APRI, aspartate transaminase to platelet ratio index; AUROC, area under the receiver operating characteristic curve; CI, confidence interval; FIB-4, Fibrosis-4; PT-INR, international normalized ratio of prothrombin time; Vs, shear wave velocity

DeLong test: vs Vs

*: $p<0.05$, †: $p<0.01$, ‡: $p<0.001$, §: $p<0.0001$

Table 3: Cut-off value and performance of Vs in predicting F2 and F3

| | Cut-off value | Sensitivity (%) | Specificity (%) | PPV (%) | NPV (%) | PLR | NLR |
|---|---|---|---|---|---|---|---|
| F2 | 1.31 | 89.4 | 53.3 | 37.3 | 94.2 | 1.91 | 0.20 |
| F3 | 1.53 | 92.9 | 69.7 | 14.0 | 99.5 | 3.07 | 0.10 |

The optimal cut-off values were determined to maximize the sum of sensitivity and specificity.

NLR, negative likelihood ratio; NVP, negative predictive value; PLR, positive likelihood ratio; PPV, positive predictive value; Vs, shear wave velocity

Table 4: Predicting factor analysis for F2≤

| | Univariate analysis | | | Multivariate analysis | | |
|---|---|---|---|---|---|---|
| | Odds Ratio | 95% CI | *P* value | Odds Ratio | 95% CI | *P* value |
| Vs | 2.38 | 1.76–3.32 | <0.001 | **2.43** | **1.63–3.79** | **<0.001** |
| Platelet count | 0.87 | 0.53–1.40 | 0.145 | | | |
| PT-INR | 1.64 | 1.07–2.68 | 0.016 | **1.93** | **1.20–3.19** | **0.006** |
| Alanine transaminase | 1.09 | 0.82–1.40 | 0.522 | | | |
| γ-Glutamyl transpeptidase | 0.95 | 0.64–1.24 | 0.727 | | | |
| Total bilirubin | 1.38 | 1.07–1.79 | 0.009 | 1.17 | 0.69–2.07 | 0.566 |
| Total bile acid | 1.59 | 1.21–2.27 | <0.001 | 1.27 | 0.77–2.37 | 0.348 |
| Total protein | 1.54 | 1.15–2.09 | 0.004 | **1.66** | **1.11–2.56** | **0.013** |
| Albumin | 0.74 | 0.56–0.97 | 0.027 | 0.95 | 0.59–1.50 | 0.828 |
| Hyaluronic acid | 1.30 | 1.00–1.73 | 0.037 | 1.13 | 0.62–1.84 | 0.647 |
| Type 4 collagen | 1.59 | 1.20–2.17 | 0.006 | 1.23 | 0.75–2.01 | 0.407 |
| APRI | 1.23 | 0.95–1.59 | 0.090 | - | | |
| FIB-4 index | 1.19 | 1.01–1.49 | 0.245 | - | | |

APRI, aspartate transaminase to platelet ratio index; CI, confidence interval; FIB-4, Fibrosis-4; PT-INR, international normalized ratio of prothrombin time; Vs, shear wave velocity

Table 5: Receiver operating characteristic (ROC) analysis of markers in predicting F2≤ categorized according to primary disease

| | Biliary atresia (n=83) | | HCV-LC (n=70) | |
|---|---|---|---|---|
| | AUROC | 95% CI | AUROC | 95% CI |
| Vs | 0.75 | 0.64–0.84 | 0.82 | 0.67–0.91 |
| Platelet count | 0.59* | 0.46–0.71 | 0.60* | 0.44–0.74 |
| PT-INR | 0.62 | 0.46–0.74 | 0.74 | 0.60–0.84 |
| Alanine transaminase | 0.40§ | 0.29–0.53 | 0.58† | 0.43–0.72 |
| γ-Glutamyl transpeptidase | 0.57* | 0.44–0.69 | 0.33§ | 0.20–0.50 |
| Total bilirubin | 0.45‡ | 0.32–0.59 | 0.69 | 0.53–0.82 |
| Total bile acid | 0.68 | 0.37–0.89 | 0.70 | 0.54–0.82 |
| Total protein | 0.52† | 0.38–0.65 | 0.68 | 0.52–0.81 |
| Albumin | 0.46§ | 0.33–0.59 | 0.65 | 0.47–0.80 |
| Hyaluronic acid | 0.58* | 0.45–0.70 | 0.71 | 0.52–0.85 |
| Type 4 collagen | 0.63 | 0.49–0.75 | 0.71 | 0.55–0.83 |
| APRI | 0.53† | 0.40–0.65 | 0.70 | 0.54–0.83 |
| FIB-4 index | 0.53† | 0.40–0.65 | 0.74 | 0.57–0.86 |

APRI, aspartate transaminase to platelet ratio index; AUROC, area under the receiver operating characteristic curve; CI, confidence interval; FIB-4, Fibrosis-4; PT-INR, international normalized ratio of prothrombin time; Vs, shear wave velocity

DeLong test: vs Vs

*: $p<0.05$, †: $p<0.01$, ‡: $p<0.001$, §: $p<0.0001$

Table 6: Receiver operating characteristic analysis of markers in predicting F2≤ categorized according to splenectomy

| | Splenectomy (+) (n=89) | | Splenectomy (-) (n=189) | |
|---|---|---|---|---|
| | AUROC | 95% CI | AUROC | 95% CI |
| Vs | 0.80 | 0.67–0.88 | 0.76 | 0.68–0.82 |
| Platelet count | 0.59* | 0.44–0.72 | 0.59‡ | 0.49–0.67 |
| PT-INR | 0.74 | 0.61–0.84 | 0.63* | 0.52–0.72 |
| Alanine transaminase | 0.68 | 0.54–0.79 | 0.47§ | 0.38–0.57 |
| γ-Glutamyl transpeptidase | 0.32§ | 0.21–0.47 | 0.54† | 0.44–0.63 |
| Total bilirubin | 0.74 | 0.59–0.84 | 0.52‡ | 0.41–0.62 |
| Total bile acid | 0.74 | 0.61–0.84 | 0.46§ | 0.37–0.56 |
| Total protein | 0.72 | 0.57–0.83 | 0.54† | 0.44–0.63 |
| Albumin | 0.75 | 0.61–0.86 | 0.44§ | 0.35–0.54 |
| Hyaluronic acid | 0.74 | 0.56–0.86 | 0.50§ | 0.40–0.59 |
| Type 4 collagen | 0.74 | 0.59–0.84 | 0.61‡ | 0.50–0.70 |
| APRI | 0.76 | 0.62–0.86 | 0.56‡ | 0.46–0.65 |
| FIB-4 index | 0.71 | 0.55–0.83 | 0.45§ | 0.35–0.55 |

APRI, aspartate transaminase to platelet ratio index; AUROC, area under the receiver operating characteristic curve; CI, confidence interval; FIB-4, Fibrosis-4; PT-INR, international normalized ratio of prothrombin time; Vs, shear wave velocity

DeLong test: vs Vs

*: $p<0.05$, †: $p<0.01$, ‡: $p<0.001$, §: $p<0.0001$