의학석사 학위논문

Prospective Comparison of Noninvasive Fibrosis Assessment to Predict Advanced Fibrosis or Cirrhosis in Non-alcoholic Fatty Liver Disease

비알코올 지방간의 간섬유화 예측을 위한 비침습적 검사의 전향적 비교

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임상의과학과 내과학

주 세 경

A thesis of the Master's degree

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Feb 2015

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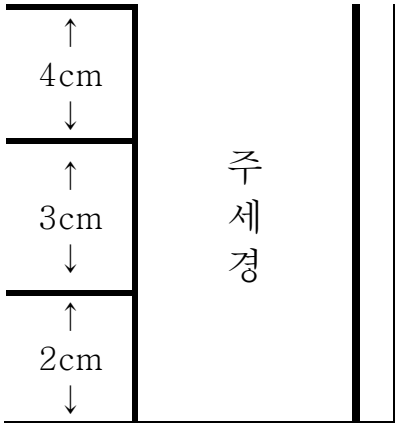
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Prospective Comparison of Noninvasive Fibrosis Assessment to Predict Advanced Fibrosis or Cirrhosis in Non-alcoholic Fatty Liver Disease

지도 교수 김 병 관

이 논문을 의학석사 학위논문으로 제출함

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주세경 의 의학석사 학위논문을 인준함

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위 원 장 미 수 (인)

Prospective Comparison of Noninvasive Fibrosis Assessment to Predict Advanced Fibrosis or Cirrhosis in Non-alcoholic Fatty Liver Disease

by

Sae Kyung Joo

A thesis submitted to the Department of Clinical Medical Sciences in partial fulfillment of the requirements for the Degree of Master of Science in Clinical Medical Sciences at Seoul National University College of Medicine

Dec 2014

Approved by Thesis Committee:

Professor Byeong Gwan Kim Chairman

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Abstract

Introduction: Non-alcoholic fatty liver disease (NAFLD) is currently the most common form of chronic liver disease. Its prevalence continues to rise, and it threatens to become a serious health problem. This study aimed to evaluate the diagnostic accuracy of noninvasive fibrosis assessment in predicting advanced fibrosis or cirrhosis in patients with NAFLD.

Methods: One hundred sixteen patients with a liver biopsy-confirmed diagnosis of NAFLD were prospectively evaluated between March 2013 and September 2014. Liver stiffness measurement (LSM) was performed by acoustic radiation force impulse (ARFI) elastography in all patients. Aspartate aminotransferase to alanine aminotransferase ratio (AAR), FIB-4 index, aspartate aminotransferase to platelet ratio index (APRI), NAFLD fibrosis score (NFS) and BARD score were calculated according to published algorithms. In order to predict advanced fibrosis or cirrhosis, diagnostic measurements of serum fibrosis indices and ARFI imaging were compared by analyzing the area under the receiver operating characteristic (AUROC) curve.

Results: The median age of the study population was 54.3 years (range, 18–78). The FIB-4 index, NAFLD fibrosis score, BARD score and LSM showed significant, positive correlations with the METAVIR stages ($P<0.001$). The LSM by ARFI had the greatest AUROC for predicting advanced fibrosis (≥F3) (0.883; 95% CI, 0.804–0.961) and cirrhosis (F4) (0.926; 95% CI, 0.848–1.000). The FIB-4 index had the good AUROC for predicting cirrhosis (F4)

(0.873; 95% CI, 0.803–0.942).

Conclusions: LSM by ARFI was a useful noninvasive assessment for predicting advanced fibrosis and cirrhosis in patients with NAFLD. In addition, the FIB-4 index exhibited acceptable diagnostic performance in the assessment of hepatic fibrosis in patients with NAFLD.

Keywords: Acoustic Radiation Force Impulse, liver stiffness, Non-alcoholic fatty liver disease, fibrosis

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CONTENTS

LIST OF TABLES AND FIGURES

LIST OF ABBREVIATIONS

NAFLD, Non-alcoholic fatty liver disease; NASH, Non-alcoholic steatohepatitis; TE, transient elastography; ARFI, acoustic radiation force impulse imaging; LS, liver stiffness; LSM, liver stiffness measurement; AAR, aspartate aminotransferase to alanine aminotransferase ratio; APRI, aspartate aminotransferase to platelet ratio index; NFS, NAFLD fibrosis score; METAVIR, META-analysis VIRus hepatitis histologic scoring system; BMI, body mass index; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma-glutamyltranspeptidase; HOMA-IR, homeostasis model assessment of insulin resistance; IFG, × impaired fasting glucose; ROI, region of interest; ANOVA, one-way analysis of variance; AUROC, area under the receiver operating characteristic; Se, sensitivity; Sp, specificity; IQR, interquartile range; PPV, positive predictive value; NPV, negative predictive value.

Introduction

Non-alcoholic fatty liver disease (NAFLD) is the most common chronic liver disease in many parts of the world, especially in developed countries. It has been estimated that about one billion individuals worldwide have NAFLD.[1] The prevalence of NAFLD had been estimated to range between 6.3 and 33%, with a median of 20% in the general population.[2] In Asia, recent reports revealed an increasing in the prevalence of NAFLD.[3, 4]

NAFLD is rapidly becoming a major health concern due to the increasing obesity epidemic and its potential to progress to liver fibrosis, cirrhosis and hepatocellular carcinoma.[5]

The spectrum of NAFLD is diverse, ranging from simple steatosis to non-alcoholic steatohepatitis (NASH), which can lead to cirrhosis. The mainstream management strategy for NAFLD is regular follow-up with risk factor modification and early detection of liver fibrosis.[6] Therefore, the prediction of liver fibrosis is very important.

To date, biopsy has been regarded as the “gold standard” for the diagnosis and assessment of liver fibrosis. However, the method is expensive, invasive, and has certain limitations. Therefore, biopsy is no longer considered the obligatory and primary screening for the diagnosis of NAFLD.[8-12]

Recently, several liver imaging techniques such as transient elastography (TE),[13] magnetic resonance imaging elastography,[14] and acoustic radiation force impulse (ARFI)[15] elastography, have been introduced for the assessment of hepatic fibrosis. Among these techniques, ARFI elastography is a novel

ultrasound-based method integrated into a conventional ultrasound system. Furthermore, ARFI enables the exact localization of the elasticity measurement site in B-mode, while TE is a blind technique with no B-mode imaging for localization. Recently, several studies on ARFI elastography have shown promising results on the correlation between hepatic fibrosis and liver stiffness (LS) and good diagnostic performance for predicting advanced fibrosis and cirrhosis.[16-18]

The aims of this study were to compare the usefulness of the fibrosis indices and to evaluate the diagnostic performance of ARFI elastography in predicting liver fibrosis in patients with NAFLD.

Materials and Methods

Study population

We prospectively evaluated 116 patients with histologically proven NAFLD, who were diagnosed by liver biopsy at the Seoul Metropolitan Government Seoul National University Boramae Medical Center between March 2013 and October 2014. The exclusion criteria included a history of excessive alcohol consumption (>20 g daily in women, >30 g daily in men); evidence of hepatitis B and C and drug-induced liver disease or other specific liver diseases; hemochromatosis; a1-antitrypsin deficiency, Wilson disease; and autoimmune liver disease. Because the increased in LS in patients with right-sided heart failure can result in the misdiagnosis of advanced fibrosis, patients with NAFLD and congestive heart failure were also excluded.[19] None of the patients had a clinical history of hepatic decompensation (ascites, bleeding from varices, encephalopathy). We included only those patients with histologically proven NAFLD. Ascites, varices and encephalopathy indicate the presence of cirrhosis, which makes invasive or non-invasive staging of fibrosis unnecessary. After excluding patients on the basis of our exclusion criteria, 116 patients were prospectively enrolled (NCT02206841). Patients with a previous diagnosis of diabetes mellitus or with a fasting glucose level > 126 mg/dL were defined as diabetic patients. Hypertension was defined as a systolic blood pressure >130/85 mmHg or need for treatment. Biochemical tests and ARFI elastography were performed on the same day as the liver biopsy. The study was approved by the Institutional Ethics Committee, and

complied with the 1975 Helsinki Declaration. Informed consent was obtained from all the study participants who were enrolled in this study.

Liver histology

Liver biopsy specimens were fixed in 4%-buffered formalin and embedded in paraffin. Two-micrometer-thick sections were stained with hematoxylin-eosin and Masson trichrome. All biopsy specimens were analyzed by an experienced pathologist who was blinded to the clinical results of the patients. Histological assessment was performed according to the Kleiner scoring system.[20] Steatosis was assessed according the number of hepatocytes with fatty degeneration: S0 = <5%, S1 = 5–33%, S2 = >33–66%, S3 = >66% of hepatocytes. Liver fibrosis was staged on a F0–F4 scale according to the Kleiner scoring system: F0, no fibrosis; F1, perisinusoidal or periportal fibrosis; F2, perisinusoidal and portal or periportal fibrosis; F3, bridging fibrosis; and F4, cirrhosis. We excluded patients with biopsy lengths that were less than 20 mm, as well as those with biopsies of fewer than eight portal tracts.

Serum fibrosis indices

The clinical and anthropometric data of the study population were collected on the same day as liver biopsy. A 12-hour overnight fasting blood sample was obtained on the day of liver biopsy to determine the serum levels of insulin, glucose, alanine aminotransferase (ALT), aspartate aminotransferase

(AST), gamma-glutamyltranspeptidase (GGT), and total bilirubin, along with prothrombin time and platelet count. The formula for calculating the homeostasis model assessment of insulin resistance (HOMA-IR) was as follows: fasting glucose (mg/dL) × fasting insulin (μU/mL)/405.[21] The AST-to-ALT ratio (AAR), and AST-to-platelet ratio index (APRI) were calculated as described elsewhere.[22-25] The FIB-4 index was calculated using the following formula: [age (years) × AST)]/[platelet counts ($\times 10^9$/l) × $ALT^{1/2}$].

The NAFLD fibrosis score (NFS) formula was = –1.675 + 0.037 – age (years) + 0.094 – body mass index (BMI) (kg/m^2) + 1.13 × impaired fasting glucose (IFG)/diabetes (yes = 1, no = 0) + 0.99 × AAR – 0.013 × platelet count ($\times 10^9$/l) – 0.66 × albumin (g/dL). Diabetes mellitus was diagnosed when fasting glucose level was ≥126 mg/dL or if the patient was treated with anti-diabetic drugs, or had IFG (defined as fasting glucose level between 100 and 125 mg/dL). In each patient with diabetes mellitus or IFG, the level of glycosylated hemoglobin (HbA1C) was determined. According to Angulo et al, a score lower than –1.455 (low cutoff) excludes advanced fibrosis, whereas a score higher than 0.676 (high cutoff) predicts advanced fibrosis. Scores between these values are defined as indeterminate.[26]

The BARD score is composed of the following 3 variables: AAR ≥ 0.8 = 2 points; BMI ≥ 28 kg/m^2 = 1 point; and presence of diabetes = 1 point. The possible score ranges from 0 to 4 points. According to the results of Harrison et al., BARD scores equaling 0 or 1 are of high (96%) negative predictive value (NPV) for advanced fibrosis.[27] The NFS and BARD score were

compared with the liver biopsy findings.

Acoustic radiation force impulse imaging

In order to measure of LS, ARFI (Acuson S2000^{TM}, Siemens AG, Germany) with the Virtual Touch tissue quantification software was used by a single experienced radiologist, who was unaware of the clinical and biochemical data. Prior to performing ARFI elastography, the gross morphologies of the liver, gall bladder, and spleen were examined using conventional ultrasonography. With real-time B-mode imaging, a 10 × 5-mm region of interest (ROI) cursor was placed on the liver parenchyma at least 3 cm below the liver capsule, without any vessel or focal liver lesion. LS measurement (LSM) was obtained at the right hepatic lobe, which was accessed through the 9–10th rib intercostal approach, with the patient in the supine position with the right arm in maximum abduction. The mean value of a series of shear wave velocities was regarded as LS and expressed in meters per second (m/s). For assessing the reproducibility of LSM by mean of ARFI elastography, a preliminary study was carried out on 50 patients with chronic liver disease who were not included in this study. Consequently, intra-observer reproducibility of 93.5% and inter-observer reproducibility of 93.2% were achieved.

Statistical analysis

Descriptive values are presented as frequency (percentage) and medians

(interquartile range, IQR). Quantitative data were analyzed using the Student *t*-test or the non-parametric Mann-Whitney *U* test. Three independent groups were compared using one-way analysis of variance (ANOVA). Qualitative data were analyzed using the chi-square test. Spearman correlation analysis was performed to assess the relationship between the METAVIR stages and each fibrosis index. The area under the receiver operating characteristic (AUROC) curve was estimated for evaluating ability to classify each parameter. Sensitivity (Se), specificity (Sp), positive predictive values (PPVs), and negative predictive values (NPVs) were calculated from the AUROC curves. Optimal cutoff LS value and serum fibrosis indices were chosen based on the highest Youden's index. The statistical significance of the differences between AUROC values was compared using the DeLong's test. In order to measure the inter-observer agreement for the histopathological assessment data; the Cohen Kappa statistic was used. Statistical analyses were performed using the IBM SPSS Statistics software package version 20.0 (IBM, Inc., Chicago, IL, USA) and R version 3.0.2 (http://www.r-project.org). P values < 0.05 were considered statistically significant.

Results

Demographic and clinical characteristics of the study population

A total of 116 patients were included in this prospective cohort study. The median age was 54.3 years (IQR, 40–65), and 42.2% of the patients were men. The median BMI was 26.8 kg/m^2 (IQR, 24.23–29.98), and the median HOMA-IR was 3.61 (IQR, 2.31–5.07). The baseline demographic, clinical, and biochemical characteristics of the study population are summarized in Table 1. The median value for LS measured by ARFI elastography was 1.13 m/s (IQR, 0.97–1.35) (Table 1).

Histological characteristics of liver biopsy

The median length of the biopsy specimens was 2.6 cm (IQR, 2.4–3.0), which was compliant with the specimen adequacy criteria. The average number of portal tracts of the biopsy specimen was 10 (range 8–24). The distribution of the METAVIR stage was as follows: F0 in 5 patients (4.3%), F1 in 56 patients (48.3%), F2 in 33 (28.4%), F3 in 10 (8.6%), and F4 in 12 (10.3%). Moderate to severe hepatic steatosis (≥33%) was present in 59.4% of the study participants (Table 1).

Table 1. Baseline characteristics of the study population

Variable	Unit	Median (IQR) or N (%)
Sex (male/female)		49 (42.2)/67 (57.8)
Age	years	54.3 (40–65)
BMI	kg/m^2	26.8 (24.23–29.98)
Diabetes		44 (37.9)
Hypertension		50 (43.1)
Fasting insulin	μU/mL	11.9 (8.48–18.3)
Fasting glucose	mg/dL	110 (95–131.75)
HOMA-IR		3.61 (2.31–5.07)
Laboratory results		
AST	IU/L	37.5 (26–60)
ALT	IU/L	41 (25–62.25)
GGT	IU/L	44.5 (24–82.25)
Total bilirubin	mg/dL	0.8 (0.6–1.1)
Cholesterol	mg/dL	179 (158.25–205.25)
Triglyceride	mg/dL	134 (87.5–179.25)
HDL	mg/dL	45 (38–54)
LDL	mg/dL	104 (84–125)
Prothrombin time	INR	1.04 (1–1.1)
Platelet	$\times 10^9/L$	227 (189–279)
Biopsy length	cm	2.6 (2.4–3.0)
Number of portal tracts		10 (8–24)
METAVIR stage		
F0		5 (4.3)
F1		56 (48.3)
F2		33 (28.4)
F3		10 (8.6)
F4		12 (10.3)
Steatosis (%)		
0		27 (23.3)
0–33		20 (17.2)
33–66		33 (28.4)
≥66		36 (31)
Serum fibrosis indices		
AAR		0.92 (0.71–1.28)
APRI		0.44 (0.29–0.76)
FIB-4		1.39 (0.87–2.29)
NFS		–1.46 (–2.57– –0.53)
BARD		
0		14 (12.1)
1		46 (39.7)
2		44 (37.9)
3		12 (10.3)
4		0
ARFI elastography		
Liver stiffness	m/s	1.13 (0.97–1.35)
Spleen measurement	mm	95.2 (86.2–105.3)

AAR, aspartate aminotransferase to alanine aminotransferase ratio; ALT, alanine aminotransferase; APRI, aspartate aminotransferase to platelet ratio index; ARFI, acoustic radiation force impulse; AST, aspartate aminotransferase; BMI, body mass index; GGT, gamma-glutamyltranspeptidase; HDL, high-density lipoprotein; HOMA-IR, homeostasis model assessment of insulin resistance; INR, international normalized ratio; IQR, interquartile range; LDL, low-density lipoprotein; NFS, NAFLD fibrosis score.

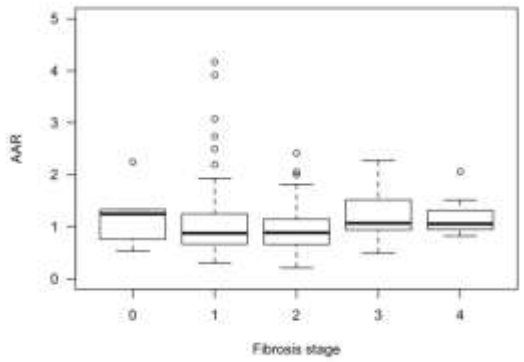
Relationship between serum fibrosis indices and histological stages

Figure 1 shows the changes in serum fibrosis indices across METAVIR stages. Positive linear correlations were reported both FIB-4 index and NFS according to METAVIR stages ($P \leq 0.001$). However, the AAR and APRI were not significantly associated with histological stages.

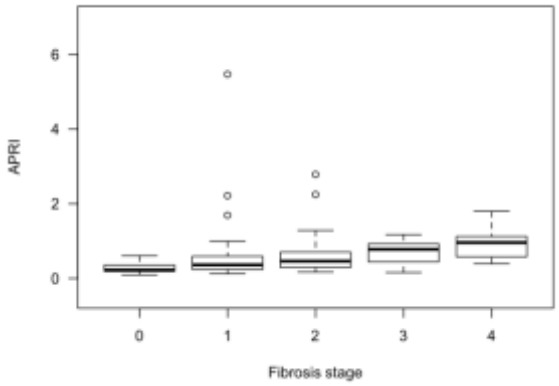
Moreover, the fasting insulin and glucose levels, and the HOMA-IR were also not significantly associated with histological stages (data not shown).

Figure 1. Boxplots showing the changes in serum fibrosis indices (A, AAR; B, APRI; C, FIB-4; D, NFS) across METAVIR stages

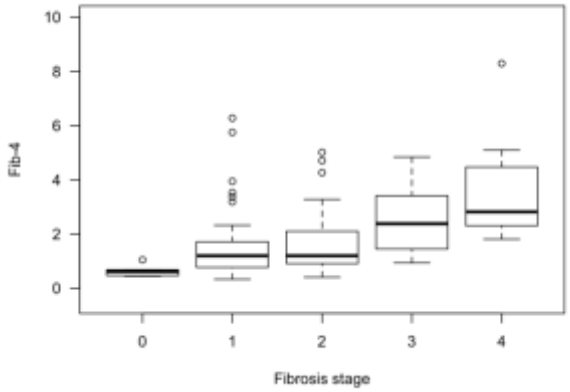
A



B


APRI
0
2
4
6
0
1
2
3
4
Fibrosis stage

C


Fib-4
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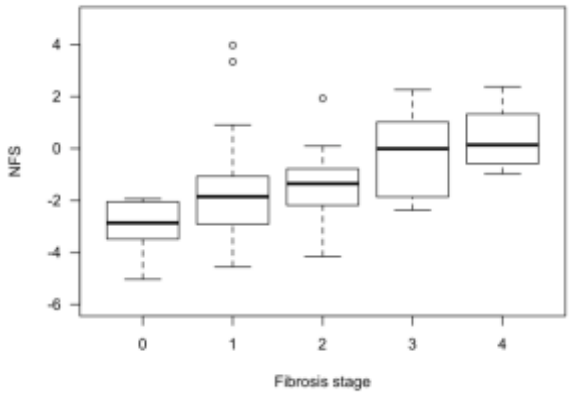
6
8
10
0
1
2
3
4
Fibrosis stage

D


NFS
-6
-4
-2
0
2
4
0
1
2
3
4
Fibrosis stage

Comparison of the diagnostic performances of serum fibrosis indices in predicting advanced fibrosis and cirrhosis

Among the serum or mechanical fibrosis indicators, FIB-4 index and NFS showed greater diagnostic accuracy in terms of prediction of advanced fibrosis and cirrhosis. The corresponding AUROC curves were then analyzed to compare the diagnostic performances of the above indices for predicting advanced fibrosis (≥F3) and cirrhosis (F4) (Fig. 2). Figures 2A and B depict the AUROCs of the previously mentioned three indices for advanced fibrosis and cirrhosis. These indices were comparable for the diagnosis of ≥F3, although FIB-4 index and NFS exhibited significantly better performance than AAR or APRI (Table 5, $P < 0.001$). For ≥F3, an optimal cutoff FIB-4 index was 1.743, with 86.36% Se and 74.19% Sp. The AUROC for the FIB-4 index was 0.826 (95% CI, 0.737−0.915; $P < 0.001$) for predicting advanced fibrosis (Table 2, Fig. 2A). The corresponding AUROC for predicting advanced fibrosis was 0.824 (95% CI, 0.729−0.920; $P < 0.001$) for NFS (Table 3, Fig. 2A).

Similarly, FIB-4 index (AUROC, 0.874; 95% CI, 0.804−0.943; $P < 0.001$) exhibited better performance for predicting cirrhosis than NFS (Table 2 and 3, Fig. 2B). The corresponding AUROC for predicting cirrhosis was 0.869 (95% CI, 0.797−0.940; $P < 0.001$) for NFS (Table 3, Fig. 2B). The optimal cutoff FIB-4 index for predicting cirrhosis was 1.792, with 100.0% Se and 72.82% Sp (Table 3).

Table 2. Diagnostic accuracy of FIB-4 for the assessment of fibrosis according to METAVIR stages

	AUROC	95% CI	Cutoff	Se (%)	Sp (%)	PPV (%)	NPV (%)
F0 vs. F1–4	0.851	0.750–0.952	1.057	69.09	100.00	100.00	12.82
F0–1 vs. F2–4	0.684	0.585–0.783	1.733	56.36	80.00	72.09	66.67
F0–2 vs. F3–4	0.826	0.737–0.915	1.743	86.36	74.19	44.19	95.83
F0–3 vs. F4	0.874	0.804–0.943	1.792	100.00	72.82	30	100.00

AUROC, area under the receiver operating characteristic; CI, confidence interval; NPV, negative predictive value; PPV, positive predictive value; Se, Sensitivity; Sp, Specificity.

Table 3. Diagnostic accuracy of NFS for the assessment of fibrosis according to METAVIR stages

	AUROC	95% CI	Cutoff	Se (%)	Sp (%)	PPV (%)	NPV (%)
F0 vs. F1–4	0.806	0.666–0.945	-1.915	65.45	100.00	100.00	11.63
F0–1 vs. F2–4	0.709	0.613–0.805	-1.440	69.09	68.33	66.67	70.69
F0–2 vs. F3–4	0.824	0.797–0.940	-0.780	77.27	80.65	48.57	93.75
F0–3 vs. F4	0.869	0.729–0.920	-0.975	100	69.9	27.91	100.00

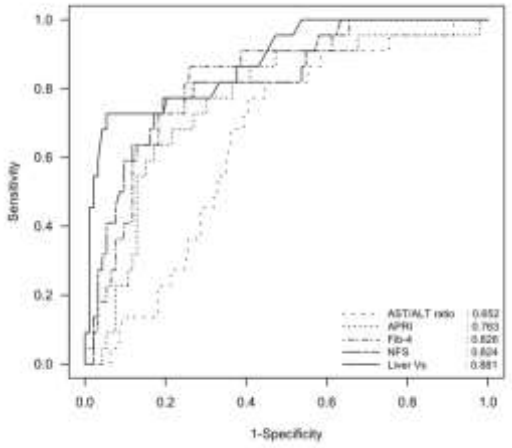
AUROC, area under the receiver operating characteristic; CI, confidence interval; NFS, NAFLD fibrosis score; NPV, negative predictive value; PPV, positive predictive value; Se, Sensitivity; Sp, Specificity.

Diagnostic performance of liver elastography according to histological staging

Briefly, figure 3 shows the median value and 95% CI of LSM according to the METAVIR stages. The median LS measured by ARFI elastography increased according to METAVIR stages ($P < 0.001$) (Fig. 3). ARFI

elastography exhibited acceptable diagnostic performance for predicting advanced fibrosis and cirrhosis (Table 5, Fig. 2A and B). The AUROCs of LSM for predicting ≥F3 and F4 were 0.881 (95% CI, 0.800−0.962) and 0.828 (95% CI, 0.845−1.000), respectively (Table 4). For ≥F3, the optimal cutoff LS value was 1.45 m/s, with 72.73% Se, 94.62% Sp, 76.19% PPV, and 93.62% NPV. The optimal cutoff value for predicting cirrhosis was 1.465 m/s, with 91.67% Se, 90.29% Sp, 52.38% PPV, and 98.94% NPV (Table 4).

Figure 2. Comparative AUROCs of AAR, APRI, FIB-4 index, NFS, and LSM by ARFI for the prediction of advanced fibrosis (A, ≥F3) and cirrhosis (B, F4)



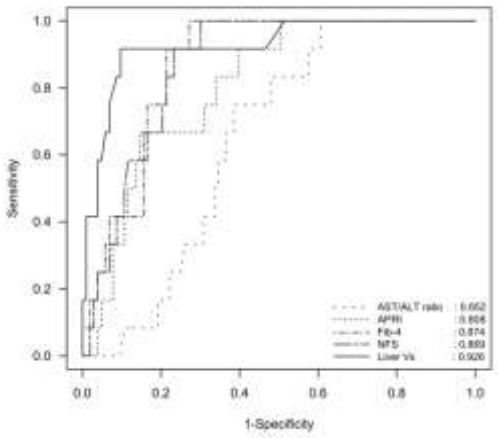


Figure 3. Distribution of liver stiffness measurements using ARFI elastography across METAVIR stages

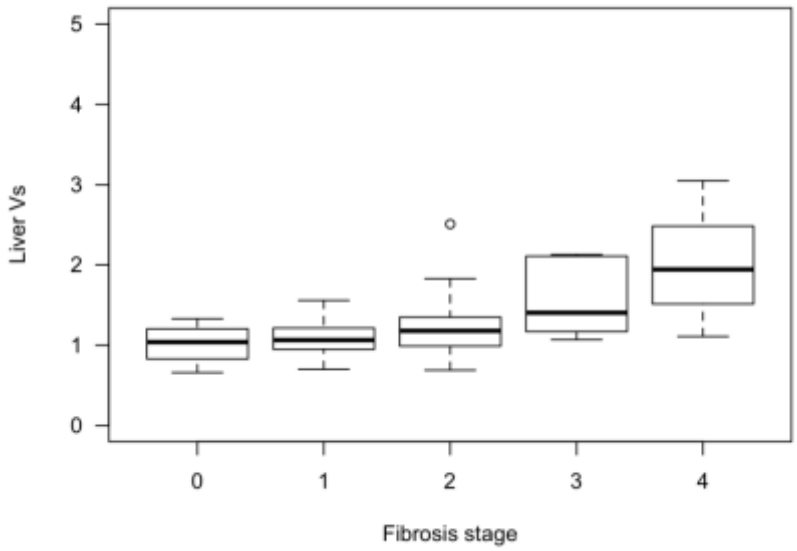


Table 4. Diagnostic accuracy of liver stiffness measurement using ARFI elastography for the assessment of fibrosis according to METAVIR stages

	AUROC	95% CI	Cutoff	Se (%)	Sp (%)	PPV (%)	NPV (%)
F0 vs. F1–4	0.651	0.361-0.941	1.085	59.46	75	98.51	6.25
F0–1 vs. F2–4	0.726	0.767–0.939	1.345	45.45	93.33	8621	65.12
F0–2 vs. F3–4	0.881	0.763–0.916	1.450	72.23	94.62	76.19	93.62
F0–3 vs. F4	0.926	0.740–0.916	1.465	91.67	90.29	52.38	98.94

AUROC, area under the receiver operating characteristic; CI, confidence interval; NPV, negative predictive value; PPV, positive predictive value; Se, Sensitivity; Sp, Specificity.

Table 5. The pairwise comparisons of prediction performance of METAVIR stages among ARFI elastography and serum fibrosis indices

METAVIR stage	Parameter1	AUROC1	Parameter2	AUROC2	*P*-value
≥ F3 vs. others	AAR	0.652	APRI	0.763	0.176
			FIB-4	0.826	0.001
			NFS	0.824	0.000
			ARFI	0.881	0.001
	APRI	0.763	FIB-4	0.826	0.209
			NFS	0.824	0.353
			ARFI	0.881	0.045
	FIB-4	0.826	NFS	0.824	0.953
			ARFI	0.881	0.232
	NFS	0.824	ARFI	0.881	0.276
F4 vs. others	AAR	0.652	APRI	0.808	0.031
			FIB-4	0.874	0.000
			NFS	0.869	0.000
			ARFI	0.926	0.000
	APRI	0.808	FIB-4	0.874	0.070
			NFS	0.869	0.300
			ARFI	0.926	0.011
	FIB-4	0.874	NFS	0.869	0.859
			ARFI	0.926	0.198
	NFS	0.869	ARFI	0.926	0.280

AAR, aspartate aminotransferase to alanine aminotransferase ratio; ALT, alanine aminotransferase; APRI, aspartate aminotransferase to platelet ratio index; ARFI, acoustic radiation force impulse; AST, aspartate aminotransferase; AUROC, area under the receiver operating characteristic; NFS, NAFLD fibrosis score.

Comparisons between liver elastography and serum fibrosis indices

As shown in figure 2 and table 5, the predictability of ARFI elastography for advanced fibrosis and cirrhosis was better than that of the FIB-4 index and NFS. To conduct pairwise comparisons between various indices' performances, the DeLong's test was performed and the results exhibited no statistical significance between ARFI elastography and other serum fibrosis indices ($P > 0.05$) (Table 5). As a result, we confirmed ARFI elastography as the best performing single noninvasive tool for the diagnosis of advanced fibrosis and cirrhosis in NAFLD with good diagnostic ability according to the AUC values.

Discussion

In this prospective evaluation of 116 Korean patients with NAFLD, our study primarily found that noninvasive indices such as APRI, AAR, FIB-4 index, and LSM using ARFI elastography had comparable diagnostic performances for advanced fibrosis and cirrhosis in patients with NAFLD. The accurate evaluation of hepatic fibrosis is crucial for making decisions regarding treatment and for predicting clinical outcomes in patients with NAFLD.[5, 28] To date, liver biopsy, has been a standard method for assessing hepatic fibrosis. Recently, several noninvasive methods for the replacement of liver biopsy have been investigated for their potential to reduce risks and medical costs.[10, 11]

A number of serum fibrosis indices have been proposed as alternatives to liver biopsy, especially for patients with viral hepatitis. These ranged from a simple test, such as platelet count measurement, to more complicated tests, including AAR, APRI, Forns index, FIB-4 index, and even patent indices (FibroTest® and ELF test®) measurement.[22-25, 30, 31] Although these tests have been validated in several clinical studies, their clinical performances compared to liver biopsy is debatable.[32-34] In the current study, we evaluated and compared the diagnostic accuracies of various serum fibrosis indices in patients with NAFLD who underwent liver biopsy. Our results also showed strong, positive correlations between serum fibrosis indices and histological fibrosis stages.

NASH is related with metabolic syndrome and glucose tolerance abnormalities.[35, 36] These factors, as well as the BMI and glucose tolerance

abnormalities, are included in NFS formula.[26] In the current study, FIB-4 index and NFS showed good diagnostic accuracy for predicting advanced fibrosis and cirrhosis. FIB-4 index is known as a simple, accurate, and inexpensive method for assessing hepatic fibrosis in patients with hepatitis C.[37] However, FIB-4 index does not include metabolic factors for calculation. Therefore, this finding may indicate strong relations between metabolic factors and the progression of liver fibrosis as indicated by Takahashi et al.[38]

In the previous study, the cutoff FIB-4 index for predicting advanced fibrosis was 3.25.[23] In our study, the cutoff FIB-4 index was 1.738 and its AUROC was 0.825. The different results between the two studies are attributable to the different disease etiologies for each group of patients. Our study included patients with NAFLD, whereas patients in the previous study had chronic hepatitis C. Therefore, it revealed FIB-4 index measurement as a useful noninvasive method for assessing advanced fibrosis and cirrhosis not only in patients with chronic hepatitis C but also in patients with NAFLD.

The NFS has been reported to have an excellent diagnostic accuracy for advanced fibrosis in patients with NAFLD. In this respect, Calès et al. reported an AUROC of 0.932 for NFS in the detection of severe fibrosis.[39] NFS had an acceptable accuracy for the diagnosis of advanced fibrosis using liver biopsy, although our data revealed a smaller AUROC for the NFS of 0.823 in the diagnostic performance of advanced fibrosis.

One of the most widely used noninvasive methods to predict hepatic fibrosis is TE-based LSM. TE is easy to use can be kept on hand in the bedside or outpatient clinic. It provides instant results and good reproducibility.[40, 41] A

recent meta-analysis demonstrated that TE was more suitable for the diagnosis of advanced fibrosis or cirrhosis than for mild fibrosis.[41] However, its accuracy might be dependent on various factors such as the acquisition rate of an adequate specimen, BMI, technical consistency, and the histological grading of fibrosis.[42] In addition, the technical drawbacks of TE can limit its use in obese patients with excessive subcutaneous fat or in cirrhotic patients with massive ascites.[43] ARFI elastography is a new imaging technique that could overcome the above technical drawbacks, and it has been recently investigated in the assessment of hepatic fibrosis.[17-18, 44,45]

Previous studies have shown a positive correlation between LSM by ARFI elastography and fibrosis stage in patients with hepatitis C.[44] However, the diagnostic performance of ARFI elastography has not been studied in patients with NAFLD. In the current study, LS measured by ARFI elastography, gradually increased in parallel with the METAVIR fibrosis stage. Moreover, ARFI elastography exhibited the highest diagnostic performance for advanced fibrosis and cirrhosis that was comparable to that of the FIB-4 index and NFS. The cutoff value (1.345 m/s) for the prediction of significant fibrosis (≥F2) was similar to the value (1.34 m/s) reported in a previous meta-analysis.[46] However, the cutoff value (1.45 m/s) for the detection of advanced fibrosis (≥F3) in the current study was lower than the value proposed in the meta-analysis (1.55 m/s).[46] In our study, the proportions of patients with advanced fibrosis and cirrhosis were different compared to those in the meta-analysis (18.9% vs. 40%).[46] Therefore, this discrepancy accounts for the different results between the studies.

This study had several limitations. First, our study utilized a cross-sectional design. Second, we did not evaluate for risk factors that might have affected the discordant results between the histological data and the noninvasive fibrosis assessment. Additional statistical analyses are required to identify the risk factors that could interfere with concordance. Third, there are other potential panels for the evaluation of liver fibrosis including the FibroTest®, and the European liver fibrosis panel, which were not examined in this study.[47, 48] Fourth, although our study population was homogenous, our results are not readily applicable to the general population given the limited sample size and the spectrum or referral bias. More generalizable results could be obtained from a larger-scale study.

In conclusion, with these caveats in mind, LSM by ARFI was an excellent imaging method for confirming advanced fibrosis and cirrhosis in patients with NAFLD. In addition, FIB-4 index and NFS were reliable markers for the assessment of advanced fibrosis and cirrhosis. Further prospective, longitudinal studies are needed to determine whether noninvasive fibrosis assessment truly reflects the dynamic changes of fibrosis in the long term.

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국문 초록

서론: 비알코올 지방간 환자에서 비침습적 섬유화 측정 방법이 간 조직검사를 대신할 수 있다는 것은 현재까지 논란의 여지가 있다. 본 연구는 비알코올 지방간 환자에서 진행된 섬유화 혹은 간 경변을 예측하기 위한 비침습적 섬유화 방법의 진단 예측 능력을 평가하고자 한다.

방법: 2013 년 3 월부터 2014 년 9 월까지 치료를 받지 않은, 116 명의 비알코올 지방간 환자를 전향적으로 등록하였다. ARFI 초음파를 이용하여 간 탄력도를 측정하였고, 동시에 조직 검사를 시행하였다. AUROC 커브를 분석하여 진행성 섬유화와 간 경변을 예측하기 위한 혈액학적 검사와 ARFI 초음파의 진단능을 비교하였다.

결과: 환자의 중위 연령은 54.3 세 (범위 18-78)였다. FIB-4 index, NFS, BARD score, ARFI 초음파를 이용한 간탄력도는 Metavir 병기에 따라 모두 강한 양의 관계를 보여 주었다. 진행성 섬유화와 간경변 예측에서 ARFI 초음파를 이용한 간 탄력도가 가장 높은 AUROC 를 보여주었으며 이 외에도 FIB-4 index 와 NFS 가 높은 AUROC 를 보여주었다.

결론: ARFI 초음파는 비알코올 지방간 환자에서 진행된 섬유화와 간경화를 예측하는데 가장 유용한 비침습적 방법이다. 또한, FIB-4

index 와 NFS 도 비알코올 지방간 환자의 간 섬유화를 측정하는데 있어 진단적으로 유용하다.

주요어: ARFI 초음파, 간 탄성도, 비알코올 지방간, 섬유화

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