Diagnostic Accuracy of Acoustic Radiation Force Impulse (ARFI) and *Wisteria floribunda* Agglutinin-Positive Mac-2-Binding Protein (WFA+-M2BP) in Patients with Chronic Liver Disease

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Background: The present study assessed and compared the diagnostic accuracy of elastography (acoustic radiation force impulse, ARFI) with that of *Wisteria floribunda* agglutinin-positive Mac-2-binding protein (WFA+-M2BP) for estimating the stage of hepatic fibrosis in chronic liver disease patients.

Material/Methods: This retrospective cross-sectional study enrolled 70 chronic liver disease patients who underwent hepatectomy for hepatic tumors. ARFI and WFA+-M2BP serum level, underlying liver disease, and laboratory data for all patients were recorded. The stage of fibrosis was determined from a surgical specimen. The area under the receiver operating characteristic (ROC) curves (AUC) was measured to compare the diagnostic accuracy.

Results: The ARFI and serum WFA+-M2BP levels had good performances for detecting severe fibrosis (≥F3). The AUC in characterization of fibrosis stage ≥F3 was 0.79 for ARFI and 0.71 for serum WFA+-M2BP levels. When comparing the diagnostic performances between ARFI and serum WFA+-M2BP levels for the severity of fibrosis stage, no significant differences were found. Then all patients were divided into 2 subgroups, the AUC for serum WFA+-M2BP levels was higher in the hepatitis C virus (HCV) subgroup than in the hepatitis B virus (HBV) subgroup when characterizing fibrosis stages ≥F3.

Conclusions: WFA+-M2BP is an accurate biomarker and is as good as ARFI in detecting severe fibrosis for chronic liver disease patients.

MeSH Keywords: Elasticity Imaging Techniques • Liver Cirrhosis • Wisteria

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Background

Cirrhosis results from chronic liver disease and is associated with the development of hepatocellular carcinoma and even death. The prognosis of chronic liver disease depends on the severity of hepatic fibrosis, which is the accumulation of extracellular matrix proteins. Because later stages of hepatic fibrosis are irreversible and lead to liver failure and death, preventing the progression of hepatic fibrosis to cirrhosis is important. Chronic liver disease management is an important issue in daily practice, and it depends on the degree of liver cirrhosis. Liver biopsy remains the criterion standard for assessment of hepatic fibrosis [1]. However, this method is expensive, needs expertise, and has the potential for procedural complications, including pain, bleeding, perforation, and even death [2]. Sampling error and interpretational variability are also potential limitations [3,4]. Thus, non-invasive methods are needed for the evaluation of hepatic fibrosis [5].

Acoustic radiation force impulse (ARFI) has been proposed in recent studies as a reliable and accurate non-invasive technique for assessing hepatic fibrosis [6]. ARFI performed better than a scoring system based on visual assessment of conventional ultrasound images by experienced radiologists in correlation with Child-Pugh scores and liver function tests and better than aspartate-to-platelet ratio in predicting severe fibrosis and cirrhosis among alcoholic liver disease patients [7].

Recently, a glycan-based immunoassay has been introduced as a convenient non-invasive method of evaluating hepatic fibrosis; it targets WFA$^+$-M2BP as a serum biomarker [8]. The feasibility of WFA$^+$-M2BP for assessing hepatic fibrosis was evaluated and some studies recommended it as an accurate method for staging hepatic fibrosis [9].

The present study aimed to assess and compare the diagnostic accuracy of WFA$^+$-M2BP and ARFI for estimating the stage of hepatic fibrosis among chronic liver disease patients.

Material and Methods

This was a retrospective cross-sectional study. Institutional Review Board approval was obtained (approval dated 20 May 2016). Between April 2016 and April 2017, 108 patients with hepatic tumors underwent hepatectomy in our institute (Changhua Christian Hospital). The inclusion criteria of our study were: (1) patients had underlying chronic liver disease, and (2) the time interval between measurement (ARFI and serum WFA$^+$-M2BP levels) and hepatectomy was less than 1 week. We enrolled 70 patients. Patient characteristics, age, sex, body mass index (BMI), underlying liver disease, and laboratory data were recorded (Table 1).

Table 1. Clinical characteristics of the 70 patients enrolled in our study.

		Total number of patients N=70 (%)	
Age (years)		64.51±10.71	
Gender	Male	58	(82.9)
	Female	12	(17.1)
BMI (kg/m^2)		24.47±3.47	
Underlying disease	HBV	33	(47.1)
	HCV	28	(40.0)
	HBV+HCV	1	(1.4)
	Alcoholic	3	(4.3)
	Cryptogenic	5	(7.1)
METAVIR Score	0	4	(5.7)
	1	13	(18.6)
	2	17	(24.3)
	3	11	(15.7)
	4	25	(35.7)
Fatty liver	0	43	(61.4)
	1	24	(34.3)
	2	3	(4.3)
AST(U/L)		76.24±87.90	
ALT(U/L)		64.11±56.68	
Bilirubin		0.93±0.45	
PLT		183.97±79.29	
APTT		34.47±3.33	
PT		11.42±1.09	
INR		1.08±0.10	
Activity	0	19	(27.1)
	1	51	(72.9)
M2BPGi (C.O.I.)		2.13±2.04	
ARFI median		2.06±0.66	

SD – standard deviation; HBV – hepatitis B virus; HCV – hepatitis C virus; AST – aspartate aminotransferase; ALT – alanine aminotransferase. Values are depicted as mean ±SD.

ARFI Elastography

ARFI was performed by experienced hepatologists who were blinded to the clinical information. A virtual touch tissue

Table 2. ARFI performance in estimation of liver fibrosis stage.

	AUC	95% CI	Sensitivity	Specificity	P value
Total; N=70					
F0–F1 *vs.* F2–F4	0.760	0.643–0.854	73.58	76.47	0.0005
F0–F2 *vs.* F3–F4	0.791	0.677–0.879	86.11	70.59	<0.0001
F0–F3 *vs.* F4	0.827	0.718–0.907	96.00	62.22	<0.0001
HBV; n=33					
F0–F1 *vs.* F2–F4	0.747	0.566–0.881	85.19	66.67	0.0919
F0–F2 *vs.* F3–F4	0.816	0.643–0.929	87.50	76.47	0.0001
F0–F3 *vs.* F4	0.813	0.640–0.927	92.31	70.00	0.0001
HCV; n=27					
F0–F1 *vs.* F2–F4	0.800	0.607–0.926	95.65	60.00	0.0130
F0–F2 *vs.* F3–F4	0.694	0.493–0.853	77.78	60.00	0.0697
F0–F3 *vs.* F4	0.856	0.671–0.959	90.91	70.59	<0.0001

AUC – area under the ROC (receiver operating characteristic) curve; CI – confidence interval. The AUC was used to measure diagnostic accuracy in each degree of hepatic fibrosis and different hepatitis virus infection status.

quantification (VTTQ) system (ACUSON S2000, Siemens AG) that uses an acoustic push pulse to generate shear waves was utilized while the patient was fasting and in supine position. The patients were asked to hold their breath. Then, the ARFI measurement was taken while patients held their breath to minimize breathing motion. The right lobe of the liver was accessed under conventional ultrasound guidance. An area of the liver free of major veins and portal tracts and having no focal pathology was selected so that the shear wave speed could be calculated. This absolute numerical value is related to the tissue stiffness within the region of interest (ROI) (1.0×0.5 cm). The results were presented in meters per second (m/s) [10,11]. The measurement of ARFI in the right lobe of the liver was performed by placing the ultrasonic probe on right intercostal spaces with light pressure, and an ROI was placed at a depth 2 cm below the liver capsule. Reliable velocity values were defined as the median of 10 valid measurements with a success rate greater or equal to 60% and an interquartile range less than 30% of the median value for the measurements [12].

WFA+-M2BP measurement

Serum samples were collected at the time of ARFI measurement. Serum WFA+-M2BP was quantified by lectin-Ab sandwich immunoassay using a fully automated immunoanalyzer (HISCL-800 Sysmex Co., Kobe, Japan) [13]. These values were successfully adjusted after every reaction during the automatic assay, and the maximum testing time was 17 min. The measured values of WFA+-M2BP conjugated to WFA were indexed with the obtained values using the following equation [8,13]:

$$\text{Cutoff index (COI)} = ([\text{WFA}^+\text{-M2BP}]_{sample} - [\text{WFA}^+\text{-M2BP}]_{negative\ control}) / ([\text{WFA}^+\text{-M2BP}]_{positive\ control} - [\text{WFA}^+\text{-M2BP}]_{negative\ control}).$$

Histologic evaluation

The results of histopathological analysis of hepatectomy specimens was used to confirm a diagnosis of hepatic fibrosis. Formalin-fixed, paraffin-embedded (FFPE) tissue samples were sectioned and stained with hematoxylin and eosin (HE) and Masson trichrome. All specimens were reviewed by an experienced hepatic pathologist blinded to clinical data. Hepatic fibrosis was stratified to stages 0–4 based on the METAVIR scoring system. Stages F0, F1, F2, F3, and F4 were defined as absent, portal fibrosis, portal fibrosis with few septa, and cirrhosis, respectively.

Statistical analysis

All continuous data are presented as mean ± standard deviation (SD). A receiver operating characteristic (ROC) curve analysis and the highest Youden index were applied to assess the optimal cutoff value for WFA+-M2BP and ARFI median (VTTQ). The area under the ROC curve (AUC) was used to measure the diagnostic accuracy in each degree of hepatic fibrosis and with different hepatitis virus infection status. The difference between 2 AUCs were compared by DeLong's test. A P value of less than 0.05 was considered to indicate statistical significance. All statistical analyses were performed using MedCalc for Windows, version 16.8.4 (MedCalc Software bvba, Ostend, Belgium).

Table 3. Serum WFA+-M2BP performance in estimation of liver fibrosis stage.

	AUC	95% CI	Sensitivity	Specificity	P value
Total; N=70					
F0–F1 *vs.* F2–F4	0.600	0.476–0.716	73.58	52.94	0.1995
F0–F2 *vs.* F3–F4	0.712	0.591–0.814	72.2	61.76	0.0006
F0–F3 *vs.* F4	0.721	0.601–0.822	84.00	55.56	0.0004
HBV; n=33					
F0–F1 *vs.* F2–F4	0.580	0.369–0.749	85.19	50.0	0.5706
F0–F2 *vs.* F3–F4	0.651	0.466–0.808	81.25	58.82	0.1404
F0–F3 *vs.* F4	0.692	0.508–0.840	84.62	65.00	0.0535
HCV; n=27					
F0–F1 *vs.* F2–F4	0.822	0.631–0.940	56.52	100.00	0.0006
F0–F2 *vs.* F3–F4	0.836	0.648–0.948	72.22	100.00	<0.0001
F0–F3 *vs.* F4	0.791	0.597–0.921	72.73	76.47	0.0012

AUC – area under the ROC (receiver operating characteristic) curve; CI – confidence interval. The AUC was used to measure diagnostic accuracy in each degree of hepatic fibrosis and different hepatitis virus infection status.

Table 4. Comparison of ARFI and WFA+-M2BP diagnostic performance in determination of liver fibrosis stage ≥F3.

		Cut-off value	AUC	95% CI	Sensitivity	Specificity	P value	Difference between areas (Mac 2 *vs.* ARFI median)	P value
Total; N=70									
F0–F2 *vs.* F3–F4	Mac 2	1.32	0.712	0.591–0.814	72.2	61.76	0.0006	0.0792	0.2057
	ARFI median	1.88	0.791	0.677–0.879	86.11	70.59	<0.0001		
HBV; n=33									
F0–F2 *vs.* F3–F4	Mac 2	0.96	0.651	0.466–0.808	81.25	58.82	0.1404	0.165	0.0816
	ARFI median	1.89	0.816	0.643–0.929	87.50	76.47	0.0001		
HCV; n=27									
F0–F2 *vs.* F3–F4	Mac 2	2.21	0.836	0.648–0.948	72.22	100.00	<0.0001	0.142	0.1782
	ARFI median	2.03	0.694	0.493–0.853	77.78	60.00	0.0697		

AUC – area under the ROC (receiver operating characteristic) curve; CI – confidence interval. The AUC values were compared by DeLong's test.

Results

Using ROC analysis, the diagnostic performance of ARFI examination in characterization of hepatic fibrosis stage is shown in Table 2. The AUC for differentiating F0–F2 from F3–F4 with ARFI examination was 0.79 (*p*<0.05) in the total group, 0.81 in the hepatitis B virus (HBV) subgroup, and 0.69 in the hepatitis C virus (HCV) subgroup. The diagnostic performance of WFA+-M2BP examination in characterization of hepatic fibrosis stage with ROC analysis is shown in Table 3. The AUC for differentiating F0–F2 from F3–F4 with the WFA+-M2BP examination was 0.71 in the total group, 0.65 in the HBV subgroup, and 0.84 in the HCV subgroup. In the HBV subgroup, WFA+-M2BP values showed no significant difference between F0–2 and F3–4 fibrosis stages. The diagnostic performance of the WFA+-M2BP examination for the HBV subgroup was inferior to that of the HCV subgroup.

ROC analysis was used to compare the diagnostic performance of ARFI examination *vs.* WFA+-M2BP values in differentiating fibrosis stages F0–F2 from F3–F4, showing no significant differences for the total group, the HBV subgroup, or HVC subgroup (Table 4). In the HBV subgroup, ARFI examination had a larger AUC than WFA+-M2BP in detecting severe fibrosis (≥F3), but the difference was not significant. In contrast, serum WFA+-M2BP examination showed a better AUC in detecting significant fibrosis (≥F3) among the HCV subgroup, but there was no significant difference between the 2 examinations.

Discussions

Our results revealed no significant diagnostic difference between serum WFA+-M2BP values (AUC, 0.71) and ARFI examination (AUC, 0.79) (p=0.206) in characterizing patients with severe fibrosis (F≥3). Kuno et al. [13] developed a rapid method using a glycan-based immunoassay, targeting WFA+-M2BP as a non-invasive biomarker for hepatic fibrosis. WFA+-M2BP now plays an important role in the evaluation of hepatic fibrosis. Toshima et al. [9] reported an AUC of 0.812 for the diagnosis of fibrosis (F≥3) using serum WFA+-M2BP values, similar to the AUC of 0.814 obtained using ARFI examination. Our results also demonstrated no significant differences in the characterization of patients with severe fibrosis (F≥3) in the HBV (*p*=0.082) and HCV (*p*=0.178) subgroups. According to our results, WFA+-M2BP could serve as a non-invasive biomarker of hepatic fibrosis, and its diagnostic performance was similar to that of ARFI elastography.

Toshima et al. [9]reported that WFA+-M2BP serum levels accurately reflect the degree of hepatic fibrosis, with an AUC of 0.81 in determining an advanced histologic stage (F≥3). In our study, the AUC of WFA+-M2BP serum levels in fibrosis stage ≥3 was only 0.71. This difference may be due to 2 reasons. In the Toshima et al. study, the number of patients with a fibrosis score of F3 was relatively small (16 patients, 8%). Another reason is that only 10% of their patients had HBV, but in our study, 47.1% of patients had HBV and 40% of patients had HCV. The AUC for the HCV subgroup was 0.836 in our study, similar to that of Toshima's study. The AUC for the HBV subgroup was only 0.65. Our results suggest that the diagnostic performance of serum WFA+-M2BP is more accurate in HCV patients than in HBV patients.

In our study, the ARFI elastography showed 86.11% sensitivity and 70.6% specificity in the diagnosis of patients with F≥3. This result is similar to that of Bota et al. [14], but the diagnostic performance of ARFI elastography was better in the HBV subgroup than in the HCV subgroup. This differs from the study by Sporea et al. [15], which found that the correlation of liver stiffness measurements assessed by ARFI elastography with

histological hepatic fibrosis was similar in patients with chronic hepatitis C *vs.* those with chronic hepatitis B. The difference might due to small case numbers in each fibrosis stage of the 2 subgroups. The diagnostic discrepancy between HBV and HCV patients needs further elucidation.

With ROC analysis in characterization of patients with severe fibrosis (≥F3), the AUC for serum WFA+-M2BP examination was 0.84 with a cutoff value of 2.21 for the HCV subgroup, and the AUC was 0.65 with a cutoff value of 0.96 for the HBV subgroup. Nishikawa et al. [16] reported the same phenomenon (COI: 3.79 for the HCV subgroup and COI: 1.79 for the HBV subgroup). The difference between the HBV and HCV subgroups may be because the pathogenic mechanisms in chronic hepatitis B infections are quite different from those in chronic hepatitis C infections. The liver histologies in the chronic hepatitis B and hepatitis C groups were also very different. Hepatitis B-related cirrhosis showed large regenerative nodules with thin fibrous septa, whereas hepatitis C-related cirrhosis showed small regenerative nodules with thick fibrous septa [17,18]. These differences may lead to different mechanisms underlying hepatic fibrosis, resulting in different cutoff values and diagnostic performances for the HCV and HBV subgroups. Further studies are needed to clarify the individual cutoff values for each etiology of chronic liver disease.

Measuring the serum WFA+-M2BP value is a rapid and non-invasive method for assessing significant hepatic fibrosis. According to our results, its diagnostic performance is as good as that of ARFI elastography. This study is simple and easily reproducible. Therefore, it might also be considered for follow-up studies during antiviral therapy of chronic liver disease. AALD guidelines recommend immediate treatment for patients with advanced liver disease (≥F3) [19]. In Taiwan, because public resources limit the ability to treat all HCV-infected patients, the patients with advanced hepatic fibrosis are regularly treated first. As a reliable non-invasive method with no need for imaging, the serum WFA+-M2BP examination might be suitable for disease characterization and therapeutic response follow-up in patients with chronic liver disease.

Our study has some limitations. First, our study design was retrospective with a small number of patients. A prospective study with a large number of cases might be needed. Second, all patients in our study underwent hepatectomy due to liver tumors. The hepatic fibrosis stages were determined according to the normal liver tissue around the tumors of surgical specimens, but there may be differences from peritumoral normal parenchyma, and the whole hepatic fibrosis condition might need to be considered. However, when the sample size is very small, surgical specimens are more accurate and reliable than liver biopsies.

Conclusions

The diagnostic accuracy of serum WFA⁺-M2BP value is comparable to that of ARFI elastography in characterization of severe hepatic fibrosis. As its measurement is rapid, simple, and non-invasive, serum WFA⁺-M2BP value might be useful in initial screening and therapeutic follow-up for patients with chronic liver disease.

Limitations

Our results may have been affected by the small sample size.

Ethics statement

All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (Changhua Christian Hospital, Taiwan) and with the Helsinki Declaration of 1964 and later versions.

Conflict of interest

None.

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