patients with AFP <20 ng/mL, when the cut-off value for AFP-L3% was set at 5%, the sensitivity and specificity of c-AFP-L3% were 7.0% and 98.5%, respectively. Those of hs-AFP-L3% were 41.5% and 85.1%, respectively. Sensitivity of hs-AFP-L3% was significantly higher than that of c-AFP-L3% ($P < 0.05$). Focusing on patients with AFP <10 ng/mL, the sensitivity of hs-AFP-L3% was 36.2%, which was still much higher than that for c-AFP-L3%. A cut-off value of 10% has been reported for diagnosis of HCC using the earlier generation methodology.[8] For this study, to maintain the specificity at 85% or more, we chose a cut-off value of 5% for hs-AFP-L3% and 10% for c-AFP-L3%.

Sensitivity with respect to tumor characteristics. Patients were classified by tumor stage (I, II, III and IV), tumor size (<2, 2–3, 3–5 and >5 cm) and tumor number (single tumor and multiple tumors). In patients with AFP <20 ng/mL, sensitivities by tumor characteristics are shown for c-AFP-L3% (cut-off 10%), hs-AFP-L3% (cut-off 5%), DCP (cut-off 40 mAU/mL) and hs-AFP-L3%-DCP combined in Table 3. Sensitivities of hs-AFP-L3% in stages I and II were 34.8% and 42.5%, respectively, whereas those of c-AFP-L3% were only 4.5% and 2.4%, respectively. Those of DCP in stage I and II were 20.2% and 57.5%, respectively. Combination of hs-AFP-L3% and DCP resulted in an improvement in sensitivity compared with hs-AFP-L3% or DCP alone. Those of the combination in stage I and II were 44.9% and 71.7%, respectively. Focusing on patients with AFP <10 ng/mL, sensitivity using the combination in stages I and II were 40.9% and 70.1%, respectively.

In patients treated by hepatectomy, 13 patients had well-differentiated HCC by postoperative pathological examination. Hs-AFP-L3% was elevated (≥5%) in four patients (30.8%). Hypervascularity of the tumor was not detected with computed tomography during hepatic arteriography, the most sensitive imaging modality to detect hypervascularity, in five patients. Hs-AFP-L3% was elevated in one of these hypovascular HCC (20.0%).

Survival rates of patients with HCC. We evaluated the significance of hs-AFP-L3% on the survival rate of HCC patients (Fig. 1). Statistical significance were not observed between the patients with high c-AFP-L3% (≥10%) and the patients with low c-AFP-L3% (<10%) ($P = 0.175$). The survival rate of patients with high hs-AFP-L3% (≥5%) was significantly lower than that of patients with low hs-AFP-L3% (<5%) by the log-rank test ($P < 0.001$). Statistical significance was not observed between the patients with high DCP (≥40 mAU/mL) and the patients with low DCP (<40 mAU/mL) ($P = 0.197$). Focusing on patients with AFP <10 ng/mL, statistical significance was still observed between the patients with high hs-AFP-L3% (≥5%) and the patients with low hs-AFP-L3% (<5%) ($P = 0.035$).

Univariate and multivariate analyses for prognostic factors for HCC. Table 4 shows the results of univariate and multivariate analyses of prognostic factors evaluated by Cox proportional hazards model in patients with AFP <20 ng/mL. The factors in the analysis were c-AFP-L3%, hs-AFP-L3%, DCP, alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin, albumin, type of treatment, tumor stage, age and gender. In the univariate analysis, the hazard ratios of hs-AFP-L3%, total bilirubin, albumin, type of treatment and tumor stage were statistically significantly high ($P = 0.001$, <0.001, 0.001, 0.001 and 0.006, respectively). Those of c-AFP-L3% and DCP were not statistically significant ($P = 0.218$ and 0.202, respectively). In the multivariate analysis, hs-AFP-L3% and non-resection were independent prognostic factors with significantly high hazard ratios ($P = 0.026$ and <0.001. respectively). For patients with AFP <10 ng/mL, hs-AFP-L3% was identified as a prognostic factor by univariate analysis ($P = 0.045$) but not by multivariate analysis ($P = 0.457$) (data not shown).

Survival rates of patients stratified by the type of treatment. In patients with AFP <20 ng/mL and classified into stages I and II, survival rates evaluated by treatment and by hs-AFP-L3% status are shown in Figure 2. All patients with any treatments ($n = 216$) are shown in Figure 2a, patients with

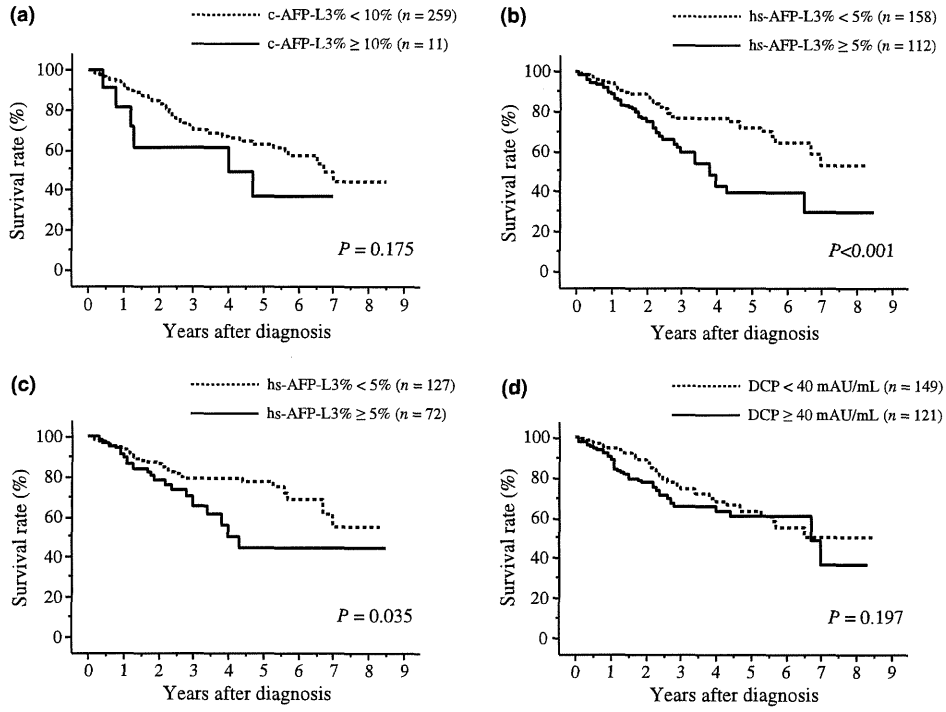

Fig. 1. Survival rates. (a) c-AFP-L3% in patients with AFP <20 ng/mL ($n = 270$), (b) hs-AFP-L3% in patients with AFP <20 ng/mL ($n = 270$), (c) hs-AFP-L3% in patients with AFP <10 ng/mL ($n = 199$), and (d) Des-gamma-carboxy prothrombin (DCP) in patients with AFP <20 ng/mL ($n = 270$). c-AFP-L3%, conventional AFP-L3%; hs-AFP-L3%, highly sensitive AFP-L3%.

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Table 4. (a) Univariate and (b) multivariate analyses for prognostic factors of hepatocellular carcinoma in patients with alpha-fetoprotein <20 ng/mL

	Variables	Hazard ratio (95% confidence interval)	*P* value
(a) Univariate analyses			
c-AFP-L3%	≥10%	1.765 (0.683–3.739)	0.218
hs-AFP-L3%	≥5%	2.195 (1.401–3.450)	0.001
DCP	≥40 mAU/mL	1.335 (0.855–2.080)	0.202
ALT	≥40 IU/L	1.132 (0.725–1.792)	0.587
AST	≥40 IU/L	1.370 (0.845–2.310)	0.207
Total bilirubin	≥1 mg/dL	2.466 (1.543–3.901)	<0.001
Albumin	<3 g/dL	2.868 (1.567–4.923)	0.001
Treatment	(LAT + TACE/ resection)	4.893 (2.876–8.832)	<0.001
Stage†	III + IV/I + II	2.111 (1.247–3.440)	0.006
Age		1.009 (0.983–1.037)	0.504
Gender	Male/Female	1.185 (0.902–1.616)	0.232
(b) Multivariate analysis			
hs-AFP-L3%	≥5%	1.697 (1.066–2.709)	0.026
Total bilirubin	≥1 mg/dL	1.575 (0.961–2.558)	0.071
Albumin	<3 g/dL	1.650 (0.878–2.930)	0.116
Treatment	(LAT + TACE/ resection)	3.627 (2.066–6.708)	<0.001
Stage†	III + IV/I + II	1.675 (0.982–2.753)	0.058

†According to TNM staging by the Liver Cancer Study Group of Japan. AFP, alpha-fetoprotein; ALT, alanine aminotransferase; AST, aspartate aminotransferase; DCP, des-gamma-carboxy prothrombin; LAT, locoregional ablative therapies; TACE, transcatheter arterial chemoembolization.

resection (n = 103) in Figure 2b, patients with LAT (n = 56) in Figure 2c and patients with TACE (n = 57) in Figure 2d. The difference in the survival rate of patients with resection was not found patients with high hs-AFP-L3% and with low hs-AFP-L3% (P = 0.813). In the case of LAT, the survival rate of patients with high hs-AFP-L3% was significantly lower than that of patients with low hs-AFP-L3% (P = 0.037). The survival rate of patients with high hs-AFP-L3% tended to be lower than that of patients with low hs-AFP-L3%, but the difference was not statistically significant in the case of TACE. The survival rate of patients with resection was significantly higher than that of patients with ablation and TACE regardless of the hs-AFP-L3% level (P = 0.002) (data not shown).

Discussion

Alpha-fetoprotein, AFP-L3% and DCP are used as markers for HCC, and their utility in the diagnosis of HCC and the evaluation of tumor progression and prognosis has been reported. Alpha-fetoprotein is the most widely used marker for monitoring HCC development. However, elevated AFP is not typically observed in patients with a small tumor or early stage HCC. Recent advances in diagnostic imaging techniques have allowed for the detection of small tumors and early stage HCC,[24–28] and the establishment of surveillance programs for HCC in the high-risk group have also contributed to diagnosis of early stage HCC.[29,30] These trends have resulted in an increase in the number of HCC patients diagnosed by imaging without elevation of AFP. Thus, HCC patients with low AFP represent the appropriate study population in a successful HCC surveillance program. Among the tumor markers, AFP-L3% is highly specific for HCC, and elevated AFP-L3% correlates with tumor progression, poor tumor differentiation and unfavorable prognosis.[8,11,31–33]

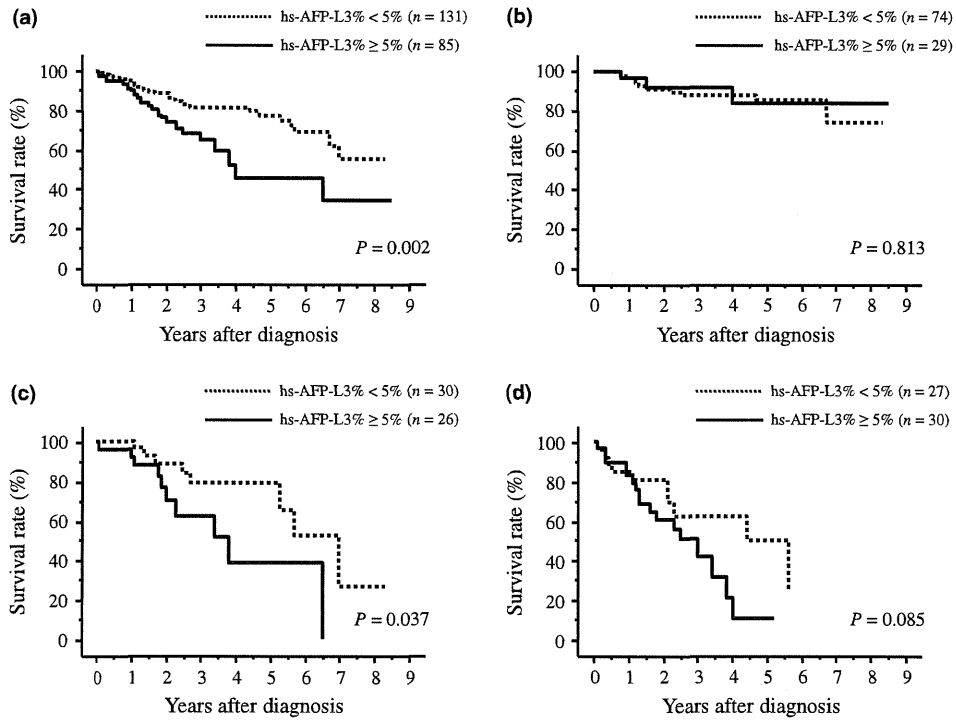

Fig. 2. Survival rates of patients stratified by the type of treatment in patients with alpha-fetoprotein (AFP) <20 ng/mL and classified into stages I and II. (a) All patients with any treatments (n = 216), (b) patients treated by surgical resection (n = 103), (c) patients treated by locoregional ablative therapies (n = 56), and (d) patients treated by transcatheter arterial chemoembolization (n = 57).

However, measurement of AFP-L3% by the conventional assay system has not always been reliable in patients with AFP <20 ng/mL due to low analytical sensitivity. Therefore, the clinical utility of conventional AFP-L3% has limited use in the diagnosis and prediction of outcome of this subpopulation. The present study focused on patients with AFP <20 ng/mL, and further, the subgroup with AFP <10 ng/mL, and revealed that the hs-AFP-L3% assay could diagnose earlier stage HCC than the c-AFP-L3 assay (cut-off 5%). The combination assay with DCP resulted in a significant improvement in diagnostic sensitivity. Parallel measurement of hs-AFP-L3% and DCP will identify additional HCC patients in the early stage because the markers are complementary for different subgroups of HCC.

Regarding prognosis, in patients with AFP <20 ng/mL, the survival rate of patients with elevated hs-AFP-L3% (≥5%) was significantly lower than that of patients with low hs-AFP-L3% (<5%). Univariate and multivariate analysis identified hs-AFP-L3% as an independent factor associated with long-term survival. Furthermore, high hs-AFP-L3% (≥5%) in the present study suggested an unfavorable prognosis, even when focusing on patients with stages I and II. In patients with stages I and II HCC treated by surgical resection, there was no statistically significant difference in survival between patients with high hs-AFP-L3% (≥5%) and those with low hs-AFP-L3% (<5%). The survival rate of patients treated by hepatic resection was much higher than that of patients with LAT or TACE. Thus, hepatic resection demonstrated favorable effects on survival compared with the other treatments, which might confound the clinical utilities of hs-AFP-L3%. In patients with AFP <10 ng/mL, hs-AFP-L3% was not identified as a prognostic factor by multivariate analysis, probably for the same reason. Although in our previous study using conventional AFP-L3% there was no difference in survival between patients with high AFP-L3% and those with low AFP-L3% in the patients treated surgically,[34] postoperative AFP-L3% has been reported as a predictive marker for recurrence and long-term survival.[35] To evaluate the prognosis of patients with resection, measurements of hs-AFP-L3% using samples after treatment should be performed.

The lower survival rate of patients with elevated hs-AFP-L3% and high rate of elevation in early stage HCC indicated that hs-AFP-L3 will be useful in identifying early stage HCC but with poorer prognosis, for which early diagnosis and treatment would be important. It may be advisable that hs-AFP-L3% should be included as a routine screening tool for HCC in the surveillance of patients at high risk of the development of HCC, together with imaging modalities.

In conclusion, the present study shows that hs-AFP-L3% was a useful marker for the diagnosis of early stage HCC in patients with AFP <20 ng/mL, and parallel measurement with DCP improved sensitivity. In addition, measurement of hs-AFP-L3% before treatment could help predict patient prognosis.

Disclosure Statement

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ORIGINAL ARTICLE—LIVER, PANCREAS, AND BILIARY TRACT

New method for assessing liver fibrosis based on acoustic radiation force impulse: a special reference to the difference between right and left liver

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Abstract
Background Virtual touch tissue quantification (VTTQ) based on acoustic radiation force impulse (ARFI) imaging has been developed as a noninvasive bedside method for the assessment of liver stiffness. In this study, we examined the diagnostic performance of ARFI imaging in 103 patients, focusing on the difference in VTTQ values between the right and left liver lobes.
Methods We evaluated VTTQ values of the right and left lobes in 79 patients with chronic liver disease who underwent histological examination of liver fibrosis and in 24 healthy volunteers. The diagnostic accuracy of VTTQ was compared with several serum markers, including hyaluronic acid, type 4 collagen, and aspartate transaminase to platelet ratio index.
Results The VTTQ values (meters per second) in the right and left lobes were 1.61 ± 0.51 and 1.90 ± 0.68, respectively, and the difference was statistically significant ($P < 0.0001$). The VTTQ values in both liver lobes were correlated significantly with histological fibrosis grades ($P < 0.001$). The standard deviations of the VTTQ values in the right lobe were significantly lower than those in the left lobe ($P < 0.001$). The area under the receiver-operating characteristic curve for the diagnosis of fibrosis ($F \geq 3$) using VTTQ values in both liver lobes was superior to serum markers, especially in the right lobe.
Conclusions VTTQ is an accurate and reliable tool for the assessment of liver fibrosis. VTTQ of the right lobe was more accurate for diagnosing liver fibrosis than in the left lobe.

Keywords Virtual touch tissue quantification · Acoustic radiation force impulse · Liver fibrosis · Transient elastography · Chronic hepatitis

Abbreviations

VTTQ	Virtual Touch^{TM} tissue quantification
ARFI	Acoustic radiation force impulse
ROC	Receiver operating characteristic
ALT	Alanine aminotransferase
LDLT	Living donor liver transplantation
APRI	Aspartate transaminase-to-platelet ratio index
HCVAb	Hepatitis C virus antibody
HBsAg	Hepatitis B virus surface antigen
m/s	Meters per second
AST	Aspartate aminotransferase
PPV	Positive predictive value
NPV	Negative predictive value
HBV	Hepatitis B virus
HCV	Hepatitis C virus

Introduction

The management of chronic liver disease depends on the degree of liver fibrosis, and therefore, the assessment of the degree of liver fibrosis is important for choosing a therapeutic strategy and for determining the prognosis [1, 2]. Liver biopsy has been the gold standard method for

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evaluating the degree of liver fibrosis [3]. However, its invasiveness, potential for life-threatening complications, and sampling errors place a heavy burden on those patients with hepatitis who require follow-up [4–6]. Therefore, noninvasive examination methods for assessing the degree of liver fibrosis, such as serum markers and transient elastography (FibroScan®, Echosens, Paris, France), have been proposed and tried [7–9].

Recently, a method based on acoustic radiation force impulse (ARFI) imaging, Virtual Touch™ tissue quantification (VTTQ), has been introduced to evaluate organ stiffness. The mechanism of VTTQ measurement exploits the phenomena whereby lower displacement magnitudes are induced in cirrhotic liver tissue compared with those induced in noncirrhotic liver tissue, as reported by Nightingale et al. [10]. VTTQ measurements can be performed during observation of a particular liver lesion with an ultrasonic probe, and measurements may be reproducible when compared with transient elastography.

In two pilot studies, it was concluded that liver VTTQ based on ARFI might be useful for the assessment of liver fibrosis in patients with chronic liver disease [11, 12]. In each study, the areas under the receiver-operating characteristic (ROC) curves for F2 were 0.82 and 0.94. However, the number of patients studied was <100, and both study groups were comprised of hepatitis patients, including hepatitis C virus (HCV). Regardless, we observed significant differences in VTTQ values between the right and left lobes of the liver, although this finding has not been reported.

To our knowledge, this is the first report to quantify liver fibrosis stiffness in both the right and left lobe of the liver by VTTQ examination in a study size >100. In this study, we compared the diagnostic accuracy of VTTQ in both the right and left lobes of the liver, using the area under the ROC curves. Furthermore, the diagnostic performance of VTTQ was compared with validated serum fibrosis markers, including the levels of hyaluronic acid and type 4 collagen, and the aspartate transaminase-to-platelet ratio index (APRI).

Patients and methods

Patients

We consecutively enrolled 103 adults, including 24 healthy volunteers (control; $n = 24$) and 79 patients with or without hepatitis who underwent hepatectomy or living donor liver transplantation (LDLT) and who were measured by VTTQ in the right and the left liver at Kyushu University Hospital. Of these, 73 patients underwent hepatic resection for hepatocellular carcinoma; 46 of these expressed the hepatitis C virus antibody (HCVAb), 7 were positive for hepatitis B virus surface antigen (HBsAg), and 3 were due to alcohol. One patient underwent hepatic resection as a donor for LDLT, and five patients underwent hepatic resection as a recipient. The study protocol conformed to the ethics guidelines of the 1975 Helsinki Declaration and was approved by our institutional review board.

Liver histology and quantification of liver fibrosis

All liver specimens were obtained by surgical resection and were fixed in formalin, embedded in paraffin wax, and stained with hematoxylin and eosin and Masson's trichrome. The fibrosis staging in all surgical specimens was determined independently by two pathologists who did not know the VTTQ values. In case of discrepancies, histological sections were simultaneously reviewed using a multi-pipe microscope to reach a consensus. Fibrosis staging was scored using the Scheuer classification [16] on a scale of 0–4 as follows: F0, no fibrosis; F1, enlarged, fibrotic portal tracts; F2, periportal or portal-portal septa but intact architecture; F3, fibrosis with architectural distortion but no obvious cirrhosis; F4, probable or definite cirrhosis.

Virtual touch tissue quantification and acoustic radiation force impulse

The VTTQ system was installed on an ACUSON model S2000 ultrasound system (Siemens Medical Solutions Inc., Ultrasound Division, Issaquah, WA). The operators were surgeons trained by Siemens Medical Solutions Inc. The VTTQ system utilizes an acoustic push pulse to generate shear waves, which pass through the liver parenchyma orthogonally to the acoustic push pulse, through a user-placed region of interest. When detection pulses interact with a passing shear wave, they reveal the wave's location at a specific time, allowing calculation of the shear wave speed. This absolute numerical value is related to the stiffness of the tissue within the region of interest [13–15], and the results are expressed in meters per second (m/s). For each patient, seven successful measurements were performed several days before surgical operations, during which the histological specimens were obtained. A total of 1442 measurements were performed in a total of 103 patients, including 721 measurements in both lobes, respectively. The measurement of VTTQ in the right lobe of the liver was performed by placing the ultrasonic probe on the right intercostal space, and in the left lobe of the liver, measurement was performed by placing the probe under the xiphoid process of the sternum at a depth from 2 to 4 cm. The median value of all measurements and the

standard deviation of all right and left VTTQ measurements for each patient were considered for analysis.

Surrogate serum markers

For all patients, blood samples were obtained on the same day that the VTTQ examination was performed and were examined in the same laboratory. The following parameters were determined: levels of hyaluronic acid, type 4 collagen, platelet count, aspartate aminotransferase (AST), alanine aminotransferase (ALT), and APRI. The APRI was calculated as follows: AST level (per upper limit of normal; 33 U/l) × 100/platelet count (10^9/l) [16, 17].

Statistical analysis

Differences between quantitative variables for paired samples were analyzed using a nonparametric test (Wilcoxon rank sum test with Bonferroni's adjustment). The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of liver stiffness optimal cutoff values for the diagnosis of liver fibrosis were calculated, as previously published [16, 17]. In addition, the diagnostic value of liver stiffness for predicting significant liver fibrosis (F1–F3) and cirrhosis (F4) was assessed by calculating the areas under the ROC curves. The ROC curve is a plot of sensitivity versus 1-specificity for all possible cutoff values. The most commonly used index of accuracy is the area under the ROC curve, where values close to 1.0 indicate high diagnostic accuracy, and 0.5 indicates a test of no diagnostic value. The optimal liver stiffness cutoff values used for the diagnosis of significant fibrosis and cirrhosis were selected based on the sensitivity, specificity, PPV, and NPV [16, 17, 19]. Statistical analysis of the differences between the areas under the ROC curves was based on the theory of generalized *U*-statistics [20]. All of the differences were considered statistically significant at $P < 0.05$.

Results

Patients and liver specimens

Patient characteristics are summarized in Table 1. The mean age of the patients (69 men and 34 women) was 66 ± 12 years. The number of healthy volunteers for the control was 24 and the etiology of hepatitis for 79 patients was classified as follows: hepatitis C (HCV; $n = 46$), hepatitis B (HBV; $n = 7$), alcohol ($n = 3$), and etiology unknown ($n = 23$). In the patients whose etiology of hepatitis was unknown, HCVAb, HBsAg, and hepatitis B core antibody were negative, and alcoholic hepatitis and nonalcoholic steatohepatitis were not diagnosed clinically.

Table 1 Patient characteristics

Characteristics	Mean ± SE
Sex (men/women)	69/34
Age (years)	66 ± 12
Etiology of hepatitis; control/HCVAb (+)/HBsAg (+)/alcohol/unknown	24/46/7/3/23
AST level (U/l)	48.0 ± 37.1
ALT level (U/l)	39.8 ± 31.5
Platelet ($\times 10^4$/μl)	16.2 ± 7.8
Hyaluronic acid (ng/ml)	176.8 ± 148.0
Type 4 collagen (ng/ml)	214.4 ± 84.1
APRI	0.43 ± 0.51
Fibrosis grade F; control/0/1/2/3/4	24/12/15/16/15/21

HBsAg hepatitis B surface antigen, *HCVAb* hepatitis C viral antibody, *AST* aspartate transaminase, *ALT* alanine aminotransferase, *APRI* aspartate transaminase-to-platelet ratio index

The fibrosis grades of the 79 surgical liver specimens were as follows: F0, $n = 12$; F1, $n = 15$; F2, $n = 16$; F3, $n = 15$; F4, $n = 21$, and control, $n = 24$.

Liver stiffness by virtual touch tissue quantification

Figure 1 shows box plots of the VTTQ values for each fibrosis stage, for VTTQ values of the right lobe of the liver (Fig. 1a) and left lobe of the liver (Fig. 1b). Liver stiffness values measured by shear wave velocity ranged from 0.74 to 2.88 m/s for the right lobe of the liver and from 0.84 to 3.83 m/s for the left lobe of the liver. The VTTQ values (right/left) in patients with fibrosis grade control ($n = 24$), F0 ($n = 12$), F1 ($n = 15$), F2 ($n = 16$), F3 ($n = 15$), and F4 ($n = 21$) were 1.15/1.41, 1.18/1.41, 1.51/1.85, 1.57/1.77, 1.85/1.88, and 2.10/2.66 m/s, respectively. There were significant correlations between the fibrosis stage and both right and left liver stiffness values ($P < 0.001$). The VTTQ value (1.61 ± 0.51) of the right lobe of the liver was lower than that of the left lobe of the liver (1.90 ± 0.68), and the difference was statistically significant ($P < 0.0001$) (Table 2). The standard deviation of VTTQ values in the right lobe (0.23 ± 0.18) was significantly lower than that of the left lobe (0.30 ± 0.17) ($P < 0.001$). The cutoff values were determined as described above [16, 17]. The optimal cutoff values (right/left) were 1.45/1.84 m/s for $F \geq 1$, 1.52/2.16 m/s for $F \geq 2$, 1.69/2.24 m/s for $F \geq 3$, and 1.79/2.38 m/s for $F \geq 4$ (Tables 3, 4). The areas under the ROC curve for the diagnosis of fibrosis types F1, F2, F3, and F4 with the right lobe VTTQ value were 0.81, 0.81, 0.85, and 0.87, respectively (Fig. 2a), and with the left lobe VTTQ value were 0.69, 0.71, 0.76, and 0.86, respectively (Fig. 2b). The area under the ROC curve for the diagnosis of fibrosis ($F \geq 1$) with the right lobe VTTQ value was significantly

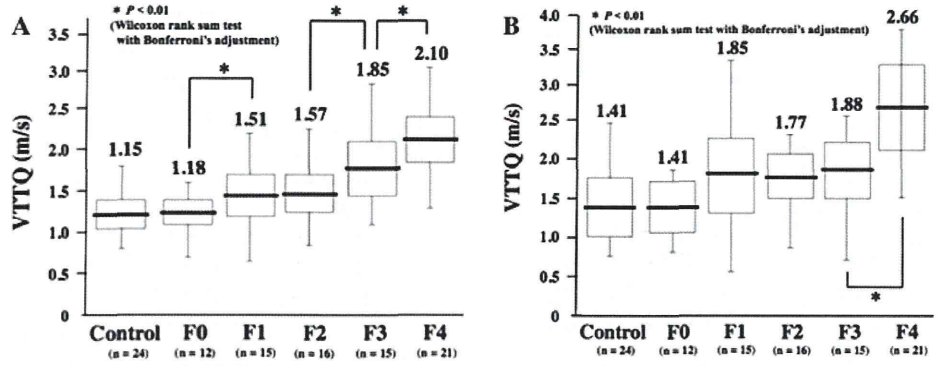

Fig. 1 Box-and-whisker plot of the VTTQ values for each fibrosis stage. Liver stiffness values measured by shear wave velocity for the right liver lobe (**a**) and for the left liver lobe (**b**). The *tops* and *bottoms* of the *boxes* represent the first and third quartiles, respectively. The *length of the box* thus represents the interquartile range, covering 50% of the values. The *line through the middle of each box* represents the median. The *error bars* show the minimum and maximum values (range). Significant correlations were found between the stage of fibrosis and liver stiffness. Statistically significant by the Wilcoxon rank sum test with Bonferroni's adjustment; $*P < 0.01$

Table 2 The difference between right and left lobe VTTQ values

$n = 103$	Right lobe VTTQ	Left lobe VTTQ	P value
VTTQ value for each patient	1.61 ± 0.51	1.90 ± 0.68	<0.0001
Standard deviation of all VTTQ values for each patient	0.23 ± 0.18	0.30 ± 0.17	<0.001

The VTTQ values of those 103 patients who were assessed in both the right and left lobes of the liver

Table 3 Liver stiffness values (right lobe)

Values (right lobe)	$F \geq 1$	$F \geq 2$	$F \geq 3$	$F \geq 4$
Optimal cutoff (m/s)	1.45	1.52	1.69	1.79
Sensitivity	0.70	0.75	0.78	0.86
Specificity	0.78	0.76	0.84	0.79
PPV	0.85	0.76	0.72	0.51
NPV	0.58	0.75	0.88	0.95

Optimal cutoff points gave the highest total sensitivity and specificity

m/s meters per second, *PPV* positive predictive value, *NPV* negative predictive value

Table 4 Liver stiffness values (left lobe)

Values (left lobe)	$F \geq 1$	$F \geq 2$	$F \geq 3$	$F \geq 4$
Optimal cutoff (m/s)	1.84	2.16	2.24	2.38
Sensitivity	0.62	0.52	0.58	0.67
Specificity	0.78	0.84	0.84	0.89
PPV	0.85	0.77	0.62	0.61
NPV	0.56	0.63	0.83	0.91

Optimal cutoff points gave the highest total sensitivity and specificity

m/s meters per second, *PPV* positive predictive value, *NPV* negative predictive value

higher than for the left lobe VTTQ value ($P < 0.05$), and the statistical significance was investigated for the diagnosis of fibrosis ($F \geq 2$ and $F \geq 3$) ($P < 0.05$). The area under the ROC curve for each point with the right lobe VTTQ was higher than with the left lobe VTTQ.

Comparison of virtual touch tissue quantification with serum markers for the diagnosis of fibrosis stage ≥3

We compared the area under the ROC curve of the serum markers (hyaluronic acid, type 4 collagen, and APRI) with that of the right lobe VTTQ values, as it was superior to the left VTTQ values for the diagnosis of all fibrosis types. The cutoff values were determined as described before. The optimal cutoff values were 1.69 m/s for the right VTTQ, 2.24 m/s for the left VTTQ, 218.0 ng/ml for hyaluronic acid, 214.0 ng/ml for type 4 collagen, and 0.24 for APRI for the diagnosis of fibrosis stage ≥3 (Table 5). The areas under the ROC curves for the diagnosis of fibrosis ($F \geq 3$) according to the right lobe VTTQ, the left lobe VTTQ, hyaluronic acid, type 4 collagen, and APRI cutoff measures were 0.85, 0.76, 0.77, 0.65, and 0.75, respectively (Fig. 3).

Discussion

This is the first report to quantify liver fibrosis in both the right and left lobes of the liver in a large population

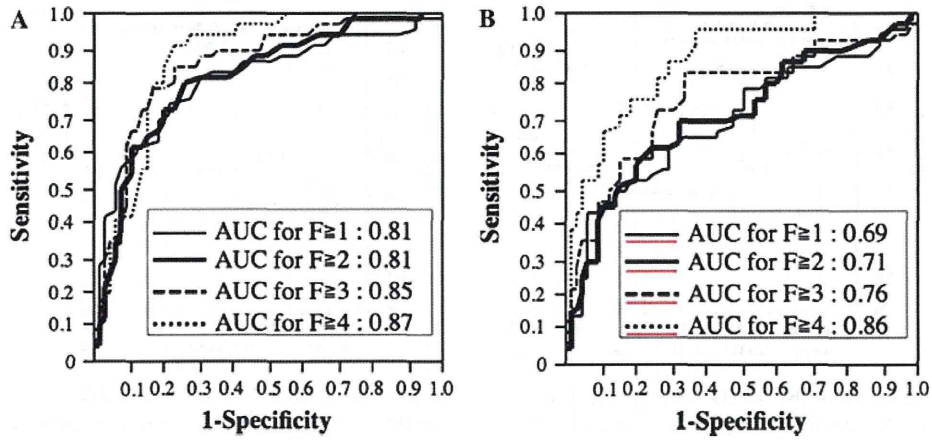

Fig. 2 Diagnostic value of the right VTTQ and the left VTTQ to assess the stage of the liver fibrosis. **a** The receiver-operating (ROC) curves by the right VTTQ for diagnosing liver fibrosis grade $F \geq 1$ (*thin black line*, area under curve = 0.81), $F \geq 2$ (*bold black line*, area under curve = 0.81), $F \geq 3$ (*dashed line*, area under curve = 0.85) and $F \geq 4$ (*dotted line*, area under curve = 0.87) are shown. **b** The receiver operating (ROC) curves by the left VTTQ for diagnosing liver fibrosis grade $F \geq 1$ (*thin black line*, area under curve = 0.69), $F \geq 2$ (*bold black line*, area under curve = 0.71), $F \geq 3$ (*dashed line*, area under curve = 0.76) and $F \geq 4$ (*dotted line*, area under curve = 0.86) are shown

Table 5 Diagnostic performance for predicting liver fibrosis ($F \geq 3$)

	VTTQ (right lobe)	VTTQ (left lobe)	Hyaluronic acid	Type 4 collagen	APRI
Optimal cutoff (unit)	1.69 (m/s)	2.24 (m/s)	218 (ng/mL)	214 (ng/mL)	0.24
Sensitivity	0.78	0.58	0.67	0.60	0.69
Specificity	0.84	0.84	0.94	0.71	0.72
PPV	0.72	0.62	0.89	0.60	0.57
NPV	0.88	0.83	0.78	0.71	0.81

VTTQ Virtual Touch™ tissue quantification, *APRI* aspartate transaminase-to-platelet ratio index, *PPV* positive predictive value, *NPV* negative predictive value

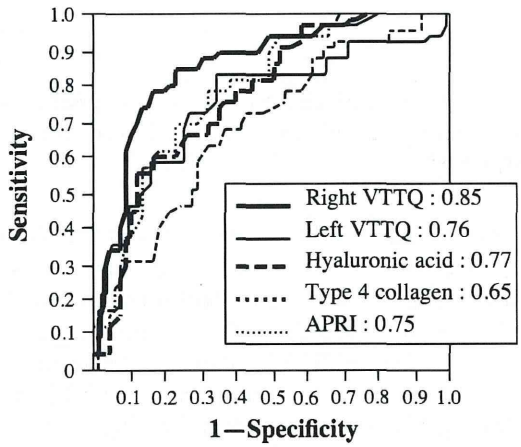

Fig. 3 Areas under the ROC curves for diagnosing fibrosis grade $F \geq 3$ by the VTTQ in the right lobe of the liver, the VTTQ in the left lobe of the liver, and serum levels of hyaluronic acid, type 4 collagen, and APRI. Shown are the ROC curves for diagnosis using the right VTTQ (*bold black line*, area under curve = 0.85), the left VTTQ (*thin black line*, area under curve = 0.76), hyaluronic acid level (*bold dashed line*, area under the curve = 0.77), type 4 collagen level (*bold dotted line*, area under the curve = 0.65), and APRI (*thin dotted line*, area under the curve = 0.75)

($n = 103$) using the VTTQ examination method. The accuracy of right and left lobe VTTQ values for diagnosing liver fibrosis grade $F \geq 3$, measured as sensitivity, specificity, PPV, and NPV, was better than serum markers of liver fibrosis, such as the levels of hyaluronic acid, type 4 collagen, and the APRI. This finding is supported by previously reported preliminary data [11, 12].

Many studies have demonstrated that measurement of liver stiffness by transient elastography using FibroScan is a valuable method for assessing liver fibrosis [16–18], and we have previously demonstrated the feasibility of FibroScan for patients with recurrent HCV after LDLT [17]. However, this approach has some limitations when compared with the VTTQ method. First, transient elastography using FibroScan is performed in a blind fashion [17]. Indeed, FibroScan includes a TM screen that shows the ultrasonographic view of the region of interest. However, only large vessels can be distinguished in the image displayed with TM mode in comparison with the B mode standard ultrasonographic image obtained by VTTQ. In most of the previous studies that employed FibroScan, the authors have stated that regions with large vessels were

avoided, and a minimal liver parenchyma thickness of 6 cm was sought to minimize the error [16–18]. Nevertheless, these types of acquisition errors can occur because this approach is performed in a blind fashion. This could be the major advantage of VTTQ over transient elastography using FibroScan for the assessment of liver fibrosis. Second, previous reports of elastography in large numbers of the patients have demonstrated that cutoff values between F2 and F3 were close [16–19]. The low predictive value of F2 and F3 could be another limitation of the FibroScan-based elastography. Patients with liver fibrosis of grade F3 or cirrhosis have greater risk of developing hepatocellular carcinoma than those with a liver fibrosis of grade F2. In a study of 2890 patients with hepatitis, Yoshida et al. [21] reported that the annual carcinogenesis rate was correlated with the stage of liver fibrosis. Whereas the annual incidence of hepatocellular carcinoma in patients with severe liver fibrosis of grade F3 was high at 5.3%, the incidence in those with moderate liver fibrosis of grade F2 was low at 1.9%. Furthermore, those patients with a liver fibrosis of grade F3 tended to progress to cirrhosis more easily than those with grade F2 disease. According to a study of 1500 patients with HCV-related chronic hepatitis, Ikeda et al. [22] reported that the progression rate to cirrhosis in patients with liver fibrosis grade F2 was 6.1%, whereas those with liver fibrosis grade F3 was very high at 50.2%, as measured at the end of the 10th year from the start of the observation. They concluded that the fibrotic change was closely correlated with the disease progression rate in patients with viral hepatitis. These findings all suggest that it is critically important to distinguish between liver fibrosis of grade F3 and F2.

Previous studies have not paid much attention to the probe position and the points of measurement when assessing liver fibrosis by VTTQ, as operators are free to measure organ stiffness at any point if they want. Nevertheless, the results of this study suggest that VTTQ measurement with the right lobe is more accurate than with the left lobe when assessing liver fibrosis. The actual values of VTTQ for the right lobe were significantly lower than for the left lobe. The standard deviation of the measured values with the right lobe was significantly lower than with the left lobe. Furthermore, the area under the ROC curve for diagnosing liver fibrosis by the right lobe VTTQ measurement was significantly higher than with the left lobe VTTQ.

Next, we examined the relationship between liver fibrosis stage and VTTQ value in the same lobe of the liver. The lobes of the 79 surgical liver specimens were as follows: right, $n = 44$ and left, $n = 37$. The areas under the ROC curve for the diagnosis of fibrosis types F1, F2, F3, and F4 in the right lobe by right VTTQ value ($n = 44$) were 0.92, 0.83, 0.86, and 0.80, and in the left lobe by left VTTQ value ($n = 37$) were 0.77, 0.71, 0.78, and 0.84, respectively. The ability to diagnose fibrosis of stage ≥ 3, which involves sensitivity, specificity, PPV, and NPV, in the right lobe by right VTTQ value were 0.88, 0.81, 0.74, and 0.92, and in the left lobe by left VTTQ value were 0.80, 0.75, 0.87, and 0.68, respectively. Considering these results, it may be concluded that the right VTTQ examination was more accurate for the diagnosis of liver fibrosis than the left VTTQ examination. The cause for this difference remains unknown. The higher standard deviation of the left lobe VTTQ values compared with the right lobe VTTQ values suggests that there may be some difficulties with measurement of VTTQ in the left lobe.

The anatomical features of the left lobe of the liver may have an influence on the measurement of VTTQ. The left lobe is surrounded by the diaphragm, stomach, and aorta, and so may be influenced by respiratory fluctuations, the presence of food in the stomach, and the pulsation of the aorta, respectively. Another factor may be the probe's position. Few studies of the measurement of liver stiffness using Fibroscan have examined the variability that is possible with different positions of the probe [23]. In almost all of the studies employing FibroScan, the described method was taken from the original description by Sandrin et al. [24], "because liver biopsies are performed on the right lobe of the liver, so were the elasticity measurements. During the acquisition, patients were lying on their backs with their right arms behind their heads. The physician first proceeded to a sonographic examination to localize the best ultrasonic imaging window between the rib bones." Recently, Ingiliz et al. [23] demonstrated two major points to consider regarding the significance of the probe position and the influence of the skin fold thickness when assessing liver stiffness. First, they showed that the anterior position of the probe should be the first choice for liver stiffness measurement using Fibroscan, as it has a higher applicability without higher variability as compared with the usual liver biopsy position. By contrast, in VTTQ measurements, organ stiffness can be measured at any point that the operators desire because the ultrasonography can be performed during acquisition, and vessels and liver parenchyma thickness are not related to the VTTQ values. Second, in their multivariate analysis, only thoracic skin fold was significantly associated with the variability of the right liver stiffness. Considering this result, the difference in the skin thicknesses between the skin on the right intercostal space and under the xiphoid process of the sternum may be due to the difference in VTTQ between the right and left lobe, and further examination is necessary.

In conclusion, VTTQ examination based on ARFI imaging is an accurate, reliable, reproducible, and noninvasive method with which to assess liver fibrosis of both the right and left lobe of the liver. This approach can be