Hirosaki Med. J. 65：199—208，2014

ORIGINAL ARTICLE

NON-INVASIVE QUANTIFICATION OF LIVER DAMAGE BY A NOVEL APPLICATION FOR STATISTICAL ANALYSIS OF ULTRASOUND SIGNALS

Yuta Yakoshi[1)], Daisuke Kudo[1)], Yoshikazu Toyoki[1)], Keinosuke Isido[1)], Norihisa Kimura[1)], Taiichi Wakiya[1)], Shingo Sakuraba[2)], Tadashi Yoshizawa[2)], Yoshiyuki Sakamoto[1)], Hiroshi Kijima[2)] and Kenichi Hakamada[1)]

Abstract **Aim**: Hepatic steatosis is a major cause of steatohepatitis and is observed in several diffuse liver diseases. We studied the efficacy of a novel ultrasound application, acoustic structure quantification (ASQ), for analyzing the amount of fat deposition in the liver parenchyma.
Methods: Forty-three patients who received a hepatectomy at our institution participated in this study. An ultrasound machine equipped with a software application for ASQ was used to detect steatosis prior to surgery. Non-tumoral liver parenchyma obtained by hepatectomy was pathologically evaluated according to the three-grade system.
Results: Histopathologically, twenty-three patients did not present steatosis, seventeen presented grade 1, and the remaining three presented grade 2 steatosis. The median "focal disturbance ratio" (FD-ratio), a representative index obtained by ASQ, decreased with the steatosis grade, from 0.157 (range: 0.039-0.410) at steatosis grade 0, 0.085 (range: 0.021-0.159) at steatosis grade 1, and 0.039 (range: 0.021-0.048) at steatosis grade 2. There were significant differences in the FD-ratio between steatosis grades 0 and 1 and between steatosis grades 0 and 2 ($p = 0.001$ and $p = 0.007$, respectively).
Conclusions: ASQ can be useful during a quantitative examination of the fat accumulation in the liver parenchyma and offers a reliable marker for the early detection of liver injury.

Hirosaki Med. J. 65：199—208，2014

Key words: Hepatic steatosis; NAFLD activity score; Ultrasound.

原　著

超音波信号の統計学的解析技術による，肝障害の非侵襲的定量

矢越雄太[1)]　工藤大輔[1)]　豊木嘉一[1)]　石戸圭之輔[1)]
木村憲央[1)]　脇屋太一[1)]　櫻庭伸悟[2)]　吉澤忠司[2)]
坂本義之[1)]　鬼島宏[2)]　袴田健一[1)]

抄録　【背景と目的】脂肪肝は，脂肪肝炎を引き起こし得る主な要因であり，種々のびまん性肝疾患において観察される．今回私達は，体外式超音波を用いた新たな解析手法である，Acoustic Structure Quantification（以下 ASQ）法を用いて肝内脂肪量を推定し，肝切除標本の病理組織学的所見と比較検討した．
【対象と方法】当科において肝切除術が施行された43症例を対象とし，術前に腹部超音波検査が全例施行され，ASQ 法による解析を行った．局所不均一パラメータである Focal disturbance ratio（以下 FD 比）を求めた．肝切除標本は，NAFLD 活動性スコアに基づいて評価し，FD 比との相関を検討した．
【結果】Steatosisスコア 0（n=23），1（n=17），2（n=3）に対し，FD 比の中央値（range）は，それぞれ0.157（0.039-0.410），0.085（0.021-0.159），0.039（0.021-0.048）であった．FD 比は，Steatosis スコア 0-1 間（$p = 0.001$）および grade 0-2 間（$p = 0.007$）で有意差を認めた．
【結語】ASQ 法により，脂肪肝の客観的かつ定量的な評価が可能となり，肝障害の早期発見における新たな指標として有用である可能性が示唆された．

弘前医学　65：199—208，2014

キーワード：脂肪肝；NAFLD 活動性スコア；超音波．

[1)] Department of Gastroenterological Surgery, Hirosaki University Graduate School of Medicine, 5 Zaifu-cho, Hirosaki, Aomori, 036-8562 Japan
[2)] Department of Pathology and Bioscience, Hirosaki University Graduate School of Medicine, 5 Zaifu-cho, Hirosaki, Aomori, 036-8562 Japan
Correspondence: K. Hakamada
Received for publication, December 25, 2013
Accepted for publication, December 27, 2013

[1)] 036-8562　青森県弘前市在府町5　弘前大学大学院医学研究科消化器外科学講座
[2)] 036-8562　青森県弘前市在府町5　弘前大学大学院医学研究科病理生命科学講座
別刷請求先：袴田健一
平成25年12月25日受付
平成25年12月27日受理

Introduction

Non-alcoholic fatty liver disease (NAFLD) is one of the most common causes of chronic liver injury in many countries around the world[1]. NAFLD includes both simple steatosis and non-alcoholic steatohepatitis (NASH)[2] that can progressively lead to cirrhosis and ultimately hepatocellular carcinoma[3-5]. Liver injury has also been observed in patients with colorectal liver metastases after systemic chemotherapy[6, 7] and it can have an undesirable effect on the perioperative course[8].

A liver biopsy is recommended to make a definitive diagnosis of steatosis and NASH[1, 9], but it carries a risk of complications[10, 11]. The accuracy of the liver biopsy for steatosis and NASH is problematic due to the inhomogeneous distribution of these diseases within the liver. Therefore, non-invasive assessment of NAFLD has been employed instead of liver biopsy.

Several methods have been reported for the quantitative analysis of steatosis and NASH, such as computed tomography (CT), magnetic resonance imaging (MRI) and ultrasound (US)[12-16]. US has been applied during steatosis screening because it is both less invasive and inexpensive relative to other modalities[12, 17, 18], but US lacks in objectivity and quantitativeness[16, 19].

Recently, acoustic structure quantification (ASQ), a novel application for ultrasound, has been developed to analyze the statistical distribution of echo patterns in the reflected signal from the parenchyma of organs, characterizing the structural changes in liver tissues[20-22]. ASQ calculates the inhomogeneity of the echo pattern of the liver and has been proposed as a useful means to exam diffuse liver disease and monitor fibrotic hepatic change during treatment[22-25]. However, the availability of ASQ for patients with NAFLD has only rarely been demonstrated.

In this study, we applied ASQ to patients intended to undergo liver surgery and evaluated its efficacy at predicting NAFLD by comparing the histopathological findings in the resected liver specimen with that of ASQ findings.

Methods

Patients

Forty-three patients who underwent liver resection at Hirosaki University School of Medicine and Hospital from April 2012 to August 2013 were enrolled in this study. The present study was approved by the ethical committee of the Hirosaki University Graduate School of Medicine, and informed consent was obtained from each included patient.

ASQ

The principle of the ASQ method[22, 24] is briefly presented below.

A region of interest (ROI) is set within the ultrasound images, and the probability density function (PDF) is calculated for the echo signal amplitude within the ROI. Therefore, the calculated PDF is known to be approximated by the Rayleigh distribution if the ROI contains only a homogeneous echo pattern and is free of any tissue structure higher than the spatial resolution level[26]. In normal liver parenchyma, the PDF is not described by the Rayleigh distribution due to the presence of the vessel walls and Glisson's capsule. The PDF in livers that contain fibrosis or cirrhosis greatly deviates from the Rayleigh distribution. However, during the progression of fatty liver, the PDF closely resembles the Rayleigh distribution.

If the examiner sets a comprehensive ROI (large-ROI) within the image, hundreds of small ROIs (small-ROI) are automatically set within the large-ROI (Figure 1A). Then, a PDF calculation and an analysis of the deviation from the Rayleigh distribution are carried out for

each of these multiple small-ROI. The chi-square test is carried out to evaluate the significance of the results, and the specific parameter "C_m^2" is defined as follows:

$$C_m^2 = \frac{\sigma_m^2}{\sigma_R^2(\mu_m)} = \left(\frac{\pi}{4-\pi}\right)\frac{\sigma_m^2}{\mu_m^2}$$

where μ and σ^2 are the average and variance of the echo signal amplitude in a small-ROI, respectively. The σ_m^2 is the variance calculated from the measured value of the signal amplitude. The $\sigma_R^2(\mu_m)$ is the approximate variance estimated from the average μ when it is supposed that the multiple small-ROI consist of homogeneous region. The C_m^2 value is the degree of deviation calculated as the ratio of σ_m^2 to $\sigma_R^2(\mu_m)$. All results for C_m^2 are plotted on an occurrence histogram (C_m^2-histogram; red line in Figure 1B). If the samples are homogeneous, the C_m^2-histogram reveals a narrow peak of 100%, and both the C_m^2 average value and the mode value are equal to 100%. However, if the samples are inhomogeneous, the C_m^2-histogram shows a broader peak, and both the C_m^2 average value and the mode value increase in relation to the inhomogeneity. In this case, the samples are considered to be either diffusely inhomogeneous or focally inhomogeneous. The latter reveals the presence of unnecessary structures (e.g., the cross section of vessels). At this point, to remove local structures from the ROIs, the ASQ software performs the procedure to recalculate C_m^2 from limited samples smaller than $\mu + 4\sigma$. Then, the recalculated C_m^2 value (rC_m^2) is smaller than C_m^2, and the C_m^2 value is sometimes even equal to 100%. If the ratio C_m^2/rC_m^2 is larger than 1.2, the result of C_m^2 is eliminated from the histogram (red line, Figure 1B) and added to the alternative histogram (blue line, Figure 1B). As a new parameter, the focal disturbance ratio (FD-ratio) is defined by the histogram. The FD-ratio is the ratio of the area under the curve (AUC) for these two histograms: FD-ratio = [AUC (blue)]/[AUC (red)]. If the samples exhibit a Rayleigh distribution for the PDF, the FD-ratio is equal to 0. Following a deviation from the Rayleigh distribution, the FD-ratio becomes larger.

Based on the above-mentioned principle, the ASQ method is designed to quantitatively evaluate slight structural changes using the FD-ratio.

Ultrasound

Ultrasonography was performed within 1 week before resection using an Aplio XG (Toshiba Medical Systems, Osaka, Japan) combined with a 3.5 MHz convex transducer (PLT-375BT). The scan mode was harmonic B-mode imaging. The display depth and transmit focus were fixed at 8 cm and 6 cm, respectively. We captured three images for analysis from the right hepatic lobe using an intercostal approach. A single ROI (600 mm^2) that included the widest portion of the hepatic parenchyma that was free of great vascular structures was positioned in each image in the vicinity of the focused area with dimensions of 30 × 20 mm. The median value of the FD-ratio was used as the final result.

Clinical and laboratory evaluation

The body weight and height of all patients were measured upon admission, and the body mass index (BMI) was calculated. Venous blood samples were obtained following 12 hours of overnight fasting, and from these samples, the platelet count (Plt), prothrombin time (PT), aspartate aminotransferase (AST), alanine transaminase (ALT), γ-glutamyl transpeptidase (γ-GTP), total bilirubin (T-Bil), total cholesterol (Chol), triglycerides (TG), and the plasma retention rate of indocyanine green at 15 minutes (ICG-R15) were analyzed in this study.

Histopathological analysis

Specimens of operative hepatic resection

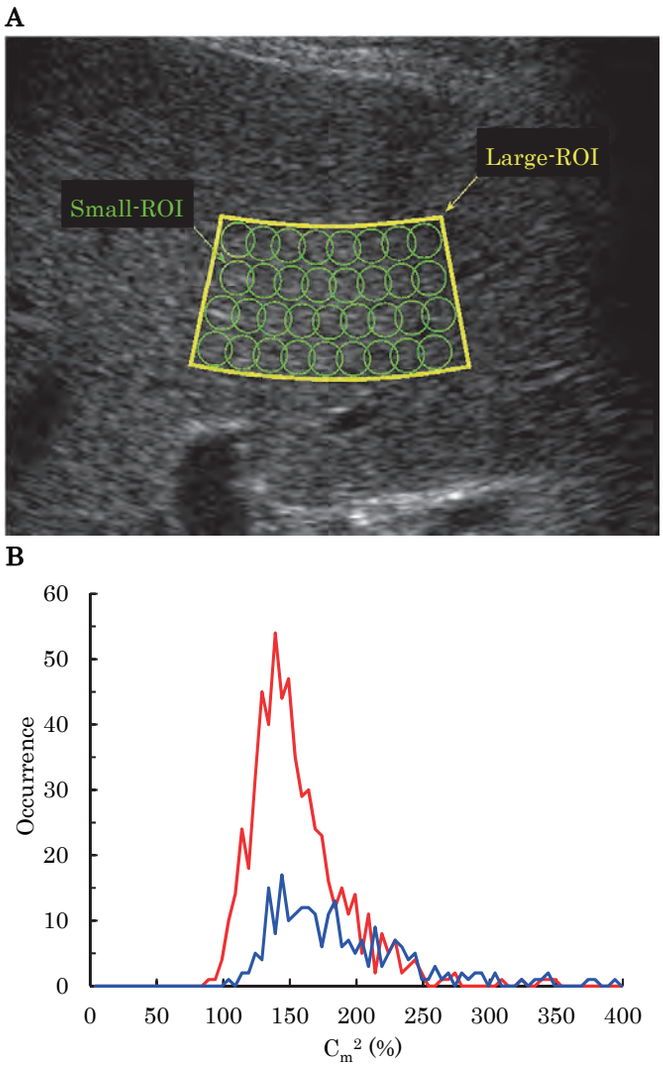

Figure 1 Schematic diagram of the region of interest (ROI) used for statistical analysis of the radio frequency signal with the acoustic structure quantification (ASQ) method, as well as the occurrence histogram.
A: A large-ROI consists of several hundred small-ROIs used to calculate the degree of deviation.
B: Results are shown in an occurrence histogram. The red line demonstrates the "diffuse inhomogeneity" and "homogeneity" of the each small-ROI, and the blue line shows the "focal inhomogeneity" of the each small-ROI.

were examined by three pathologists under blinded review, and non-tumorous tissue was evaluated in all samples from the patients. The NAFLD activity score (NAS) established by Kleiner et al.[27] was used to grade NASH, and the degree of steatosis, lobular inflammation, and hepatocellular ballooning were separately assessed. The degree of necroinflammatory activity and fibrosis of NASH was scored according to the Brunt classification[28]. The inflammatory activity and fibrosis of chronic hepatitis was assessed in accordance with the new Inuyama classification[29]. The severity of sinusoidal dilatation was scored by the method of Rubbia-Brandt[30].

Statistical analysis

Each histopathological finding was regarded as the reference standard. PASW Statistics ver. 18.0 software (SPSS Inc., Chicago, USA)

Table 1 Characteristics of all patients

	n	(%)
Age (years, mean ± S.D.)	64.1 ± 11.1	
Gender		
Male	31	(71.1)
Female	12	(28.9)
Condition		
HBV	12	(26.7)
HCV	5	(11.1)
Medication for		
Diabetes mellitus	6	(15.6)
Hypertension	5	(13.3)
Hyperlipidemia	3	(8.9)
Diagnosis		
Colorectal liver metastases	22	(48.9)
Hepatocellular carcinoma	11	(24.5)
Adenocarcinoma of the biliary tree	10	(22.2)
Operation		
Anatomical	15	(37.8)
Non-anatomical	22	(48.9)
Combined	6	(13.3)

S.D. = standard deviation.

was used to carry out all statistical analyses. The Kruskal-Wallis test was used to test the statistical significance of inter-group differences from the clinical data and histopathological findings. When a significant difference was detected using the Kruskal-Wallis test, we compared the median values of all groups using the Mann-Whitney U test. The Spearman's rank correlation coefficient was used to compare the histopathological findings with the FD-ratios. In all tests, $p < 0.05$ was regarded as a statistically significant difference.

Results

Patient characteristics

The study group consisted of 31 men and 12 women with a mean age (± standard deviation) of 64.1 (± 11.1) years. The other characteristics of all patients are summarized in Table 1.

Comparison of histopathological findings and clinical data

There were significant differences in FD-ratio among steatosis grade of NAS, but FD-ratio was not significantly correlated to the other histopathological findings (Table 2). The NAS presented a steatosis grade 0 (n = 23), 1 (n = 17), 2 (n = 3), or 3 (n = 0). There were significant differences in the FD-ratio, LS-ratio, BMI, and γ-GTP ($p < 0.001$, $p = 0.023$, $p = 0.021$, and 0.027, respectively) according to the steatosis grade of NAS (Table 3). The FD-ratio and BMI showed a stepwise change with the steatosis grade of NAS. The graphic chart of C_m^2 obtained by ASQ corresponds to the steatotic change in the liver, i.e., diffuse homogeneity in the each small-ROI yields a thinner red line, and a reduction of the focal inhomogeneity among the numerous ROIs lowers the peak of the blue line (Figure 2).

Relationship between the histological findings and clinical data

Because the FD-ratio results differed significantly depending on the steatosis grade, we compared the median value of all groups

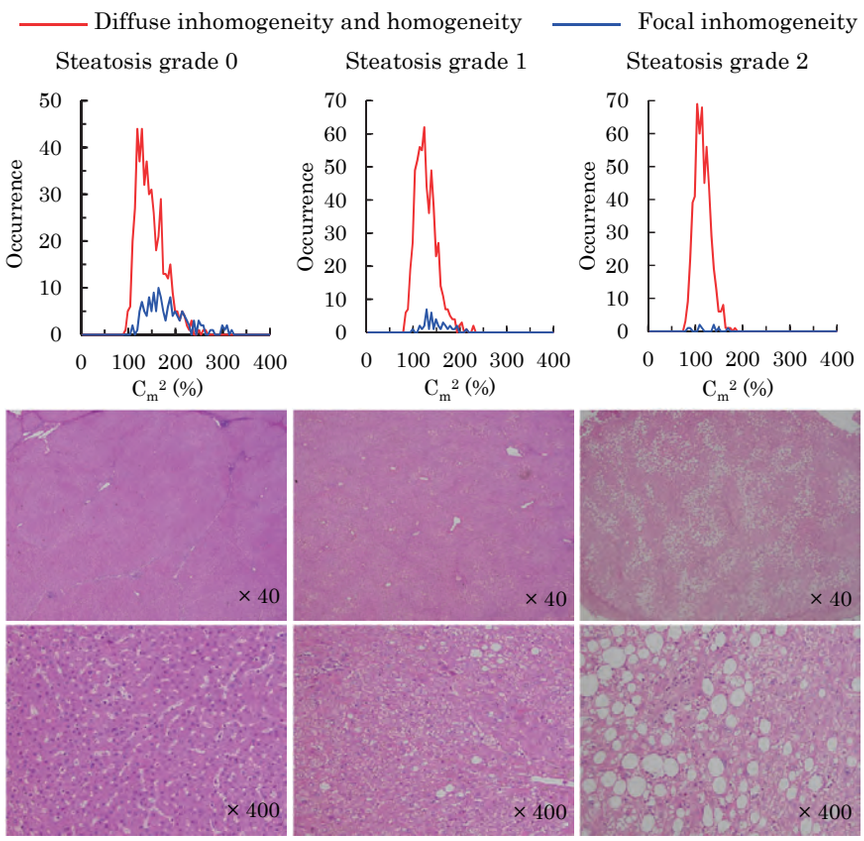

Figure 2 The occurrence histogram and microscopic evaluation of the liver parenchyma, corresponding to the steatosis grade of the NAFLD activity score.

according to the steatosis grade. The median FD-ratio decreased with the steatosis grade, from 0.157 (range: 0.039-0.410) at steatosis grade 0, 0.085 (range: 0.021-0.159) at steatosis grade 1, and 0.039 (range: 0.021-0.048) at steatosis grade 2 (Table 3). There were significant differences in the FD-ratio between steatosis grades 0 and 1 and between steatosis grades 0 and 2 ($p = 0.001$ and $p = 0.007$, respectively), although no significant difference was observed between steatosis grades 1 and 2 ($p = 0.050$) (Figure 3).

The Spearman's rank correlation coefficient revealed significant negative correlation between the steatosis grade and the FD-ratio ($r = -0.608$, $p < 0.001$).

Discussion

In the present study, we found the strongest correlation between the FD-ratio obtained by ASQ and histopathological steatosis grade of NAFLD activity score (NAS) among patients regardless of background factors, and serological liver function tests. These results suggest that ASQ analysis of the liver and its outputs reliably detect steatosis by calculating the statistical deviation of ultrasound imaging.

Although ultrasonography is perceived to be highly sensitive and reliable manifestations of fatty liver, quantitative method to examine the severity of lipid accumulation in the human liver has not yet been reported. Here, we have demonstrated that ASQ can be used to detect the degree of steatosis corresponding to the

Table 2 Focal disturbance ratio with histopathological findings

Histopathological score		n	(%)	FD-ratio median	FD-ratio (range)	p value
Sinusoidal dilatation	0	6	(14.0)	0.100	(0.021-0.191)	
	1	25	(58.1)	0.112	(0.021-0.410)	0.756
	2	12	(27.9)	0.120	(0.039-0.354)	
Inuyama classification						
Inflammation	1	27	(62.8)	0.089	(0.021-0.246)	0.088
	2	16	(37.2)	0.133	(0.048-0.410)	
Fibrosis	0	17	(39.5)	0.129	(0.021-0.246)	
	1	17	(39.5)	0.081	(0.026-0.194)	
	2	3	(7.0)	0.146	(0.048-0.298)	0.298
	3	1	(2.3)	0.125		
	4	5	(11.6)	0.134	(0.069-0.410)	
Brunt classification of NASH						
Necroinflammation	1	26	(60.5)	0.104	(0.021-0.298)	
	2	11	(25.6)	0.125	(0.026-0.189)	0.888
	3	6	(14.0)	0.108	(0.039-0.410)	
Fibrosis	1	29	(67.4)	0.096	(0.021-0.246)	
	2	8	(18.6)	0.115	(0.026-0.298)	0.486
	3	1	(2.3)	0.125		
	4	5	(11.6)	0.134	(0.069-0.410)	
NAS	0	1	(2.3)	0.180		
	1	11	(25.6)	0.139	(0.061-0.298)	
	2	19	(44.2)	0.096	(0.021-0.410)	0.043*
	3	9	(20.9)	0.132	(0.052-0.191)	
	4	3	(7.0)	0.039	(0.021-0.048)	
Lobular inflammation	0	1	(2.3)	0.180		
	1	25	(58.1)	0.089	(0.021-0.298)	0.358
	2	14	(32.6)	0.140	(0.039-0.410)	
	3	3	(7.0)	0.115	(0.069-0.191)	
Hepatocyte ballooning	0	40	(93.0)	0.116	(0.021-0.410)	0.215
	1	3	(7.0)	0.039	(0.021-0.162)	
Steatosis	0	23	(53.5)	0.157	(0.039-0.410)	
	1	17	(39.5)	0.085	(0.021-0.159)	< 0.001*
	2	3	(7.0)	0.039	(0.021-0.048)	

FD-ratio = focal disturbance ratio, NASH = nonalcoholic steatohepatitis, NAS = non-alcoholic fatty liver disease activity score.
* $p < 0.05$, Kruskal-Wallis test.

histopathological steatosis grade in patients who have received hepatectomy. Difficulties in making a diagnosis using the usual methods might arise from a lack of objectivity and quantitativeness[16, 19]; however, ASQ outputs a numerical value and provide a quantitative assessment of the liver tissue according to the lipid deposition. As this novel method is non-invasive, inexpensive, and amenable to repeated use, analysis by ASQ could be a prop for detecting the severity of hepatic steatosis and the follow up of patients with NAFLD.

During the application of ASQ, the ultrasound signals from the numerous small-ROI are analyzed respectively to ameliorate the analytical precision, and "diffuse inhomogeneity" and "focal inhomogeneity" are postulated in each small-ROI. If a large amount of fat accumulates in the liver, the parenchymal image will cause the deviation of signal intensity in the small-ROIs to be homogeneous, and the proportion of "inhomogeneous" small-ROIs will decrease.

Recently, it has been reported that certain types of chemotherapy also lead to fat

Table 3 Physical and biochemical characteristics of patients with steatosis

	Steatosis grade of NAS			
	0 (n = 23)	1 (n = 17)	2 (n = 3)	p value
BMI (kg/m^2)	21.22 (16.98-28.91)	22.66 (17.22-27.68)	25.03 (24.68-26.80)	0.021*
Plt (×10^4/mL)	17.5 (8.0-55.2)	17.9 (5.3-27.2)	17.3 (17.2-21.5)	0.879
PT (%)	97 (73.0-115.0)	99 (65.0-109.0)	91 (79.0-97.0)	0.209
AST (IU/L)	31 (10.0-54.0)	26 (13.0-83.0)	38 (35.0-41.0)	0.283
ALT (IU/L)	30 (12.0-66.0)	21 (8.0-168.0)	48 (35.0-50.0)	0.113
γ-GTP (IU/L)	81 (16.0-408.0)	38 (17.0-223.0)	92 (67.0-98.0)	0.027*
T-Bil (mg/dL)	0.6 (0.20-1.30)	0.5 (0.30-1.00)	0.5 (0.40-0.50)	0.667
Chol (mg/dL)	179 (109.0-252.0)	185 (134.0-242.0)	198 (170.0-231.0)	0.646
TG (mg/dL)	104 (60.0-227.0)	96 (56.0-240.0)	166 (82.0-203.0)	0.464
ICG-R15 (%)	14 (3.0-47.0)	10 (3.0-39.0)	8 (6.0-22.0)	0.878
FD-ratio	0.157 (0.039-0.410)	0.085 (0.021-0.159)	0.039 (0.021-0.048)	< 0.001*

Data are median value (range) measured in the current study population.
NAS = non-alcoholic fatty liver disease activity score, BMI = body mass index, Plt = platelet count, PT = prothrombin time, AST = aspartate aminotransferase, ALT = alanine transaminase, γ-GTP = γ-glutamyl transpeptidase, T-Bil = total bilirubin, Chol = total cholesterol, TG = triglycerides, ICG-R15 = indocyanine green retention rate at 15 min, FD-ratio = focal disturbance ratio
* $p < 0.05$, Kruskal-Wallis test.

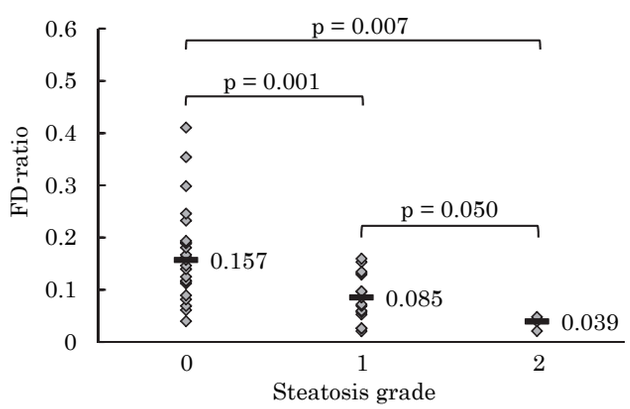

Figure 3 Comparison between the steatosis grade and the focal disturbance ratio (FD-ratio).
The median FD-ratios of all groups were compared using the Mann-Whitney U test. There were significant differences in the FD-ratio between steatosis grades 0 and 1 and between steatosis grades 0 and 2. The Spearman's rank correlation coefficient revealed a highly significant negative correlation between the steatosis grade and the FD-ratio ($r = -0.608$, $p < 0.001$).

deposition in the liver[7]. Therefore, detecting the magnitude of steatosis is thought to be increasingly important as a means of prophylaxis of the progression to liver cirrhosis and for monitoring the therapeutic efficacy in patients with chronic liver disease in general rather than simply NAFLD.

In this study, ASQ demonstrated the ability

to detect the presence or absence of steatosis. This application of ASQ might also be useful for choosing the optimal timing for hepatectomy in patients with colorectal liver metastases during systemic chemotherapy, as this combination of treatments can involve chemotherapy-associated steatosis/steatohepatitis and severe damage in the remnant liver by the chemotherapy, leading to morbidity and mortality[7, 31]. In addition, ASQ will be available to ensure proper selection of donors for liver transplants, health screening, and so on, utilizing its non-invasive and inexpensive characteristics.

In conclusion, a novel analytical method that is available using conventional ultrasonography is useful to quantitatively examine the fat accumulation in the liver parenchyma. Furthermore, it can be a reliable index for the early detection of diffuse liver disease.

References

1) Angulo P. Nonalcoholic fatty liver disease. N Engl J Med 2002;346:1221-31.

2) Ludwig J, Viggiano TR, McGill DB, Oh BJ. Nonalcoholic steatohepatitis: Mayo Clinic experiences with a hitherto unnamed disease. Mayo Clin Proc 1980;55:434-8.

3) Wanless IR, Lentz JS. Fatty liver hepatitis (steatohepatitis) and obesity: an autopsy study with analysis of risk factors. Hepatology 1990;12: 1106-10.

4) Nair S, Mason A, Eason J, Loss G, Perrillo RP. Is obesity an independent risk factor for hepatocellular carcinoma in cirrhosis? Hepatology 2002;36:150-5.

5) Adams LA, Lymp JF, St Sauver J, Sanderson SO, Lindor KD, Feldstein A, Angulo P. The natural history of nonalcoholic fatty liver disease: a population-based cohort study. Gastroenterology 2005;129:113-21.

6) Fernandez FG, Ritter J, Goodwin JW, Linehan DC, Hawkins WG, Strasberg SM. Effect of steatohepatitis associated with irinotecan or oxaliplatin pretreatment on resectability of hepatic colorectal metastases. J Am Coll Surg 2005;200:845-53.

7) Vauthey JN, Pawlik TM, Ribero D, Wu TT, Zorzi D, Hoff PM, Xiong HQ, et al. Chemotherapy regimen predicts steatohepatitis and an increase in 90-day mortality after surgery for hepatic colorectal metastases. J Clin Oncol 2006;24:2065-72.

8) Vetelainen R, van Vliet A, Gouma DJ, van Gulik TM. Steatosis as a risk factor in liver surgery. Ann Surg 2007;245:20-30.

9) Bravo AA, Sheth SG, Chopra S. Liver biopsy. N Engl J Med 2001;344:495-500.

10) Kleiner DE, Brunt EM, Van Natta M, Behling C, Contos MJ, Cummings OW, Ferrell LD, et al. Design and validation of a histological scoring system for nonalcoholic fatty liver disease. Hepatology 2005;41:1313-21.

11) Ratziu V, Charlotte F, Heurtier A, Gombert S, Giral P, Bruckert E, Grimaldi A, et al. Sampling variability of liver biopsy in nonalcoholic fatty liver disease. Gastroenterology 2005;128:1898-906.

12) Ricci C, Longo R, Gioulis E, Bosco M, Pollesello P, Masutti F, Croce LS, et al. Noninvasive in vivo quantitative assessment of fat content in human liver. J Hepatol 1997;27:108-13.

13) Park SH, Kim PN, Kim KW, Lee SW, Yoon SE, Park SW, Ha HK, et al. Macrovesicular hepatic steatosis in living liver donors: use of CT for quantitative and qualitative assessment. Radiology 2006;239:105-12.

14) Meisamy S, Hines CD, Hamilton G, Sirlin CB, McKenzie CA, Yu H, Brittain JH, et al. Quantification of hepatic steatosis with T1-independent, T2-corrected MR imaging with spectral modeling of fat: blinded comparison with MR spectroscopy. Radiology 2011;258:767-75.

15) Saadeh S, Younossi ZM, Remer EM, Gramlich T, Ong JP, Hurley M, Mullen KD, et al. The utility of radiological imaging in nonalcoholic fatty liver disease. Gastroenterology 2002;123:745-50.

16) Schwenzer NF, Springer F, Schraml C, Stefan N, Machann J, Schick F. Non-invasive assessment

and quantification of liver steatosis by ultrasound, computed tomography and magnetic resonance. J Hepatol 2009;51:433-45.

17) Saverymuttu SH, Joseph AE, Maxwell JD. Ultrasound scanning in the detection of hepatic fibrosis and steatosis. Br Med J (Clin Res Ed) 1986;292:13-5.

18) Palmentieri B, de Sio I, La Mura V, Masarone M, Vecchione R, Bruno S, Torella R, et al. The role of bright liver echo pattern on ultrasound B-mode examination in the diagnosis of liver steatosis. Dig Liver Dis 2006;38:485-9.

19) Strauss S, Gavish E, Gottlieb P, Katsnelson L. Interobserver and intraobserver variability in the sonographic assessment of fatty liver. Am J Roentgenol 2007;189:W320-3.

20) Yamaguchi T, Hachiya H, Kamiyama N, Ikeda K, Moriyasu N. Estimation of characteristics of echo envelope using RF echo signal from the liver. Jpn J Appl Phys 2001;40:3900.

21) Yamaguchi T, Hachiya H, Kamiyama N, Moriyasu F. Examination of the spatial correlation of statistics information in the ultrasonic echo from diseased liver. Jpn J Appl Phys 2002;41:3585.

22) Toyoda H, Kumada T, Kamiyama N, Shiraki K, Takase K, Yamaguchi T, Hachiya H. B-mode ultrasound with algorithm based on statistical analysis of signals: evaluation of liver fibrosis in patients with chronic hepatitis C. Am J Roentgenol 2009;193:1037-43.

23) Nakajima S, Shibuya K, Kamiyama N, Sumino Y. Comparison of ultrasound colored image views produced by application of statistical analysis of radio-frequency signals and histological findings in patients with chronic hepatitis C. J Med Ultrasonics 2010;37:51-8.

24) Kuroda H, Kakisaka K, Kamiyama N, Oikawa T, Onodera M, Sawara K, Oikawa K, et al. Non-invasive determination of hepatic steatosis by acoustic structure quantification from ultrasound echo amplitude. World J Gastroenterol 2012;18:3889-95.

25) Ricci P, Marigliano C, Cantisani V, Porfiri A, Marcantonio A, Lodise P, D'Ambrosio U, et al. Ultrasound evaluation of liver fibrosis: preliminary experience with acoustic structure quantification (ASQ) software. Radiol Med 2013;118:995-1010

26) Burckhardt CB. Speckle in ultrasound B-mode scans. IEEE Trans Sonics Ultrason 1978;25:1-6.

27) Kleiner DE, Brunt EM. Nonalcoholic fatty liver disease: pathologic patterns and biopsy evaluation in clinical research. Semin Liver Dis 2012;32:3-13.

28) Brunt EM, Janney CG, Di Bisceglie AM, Neuschwander-Tetri BA, Bacon BR. Nonalcoholic steatohepatitis: a proposal for grading and staging the histological lesions. Am J Gastroenterol 1999;94:2467-74.

29) Ichida F, Tsuji T, Omata M, Ichida T, Inoue K, Kamimura T, Yamada G, et al. New Inuyama classification; new criteria for histological assessment of chronic hepatitis. Int Hepatol Commun 1996;6:112-9.

30) Rubbia-Brandt L, Audard V, Sartoretti P, Roth AD, Brezault C, Le Charpentier M, Dousset B, et al. Severe hepatic sinusoidal obstruction associated with oxaliplatin-based chemotherapy in patients with metastatic colorectal cancer. Ann Oncol 2004;15:460-6.

31) Nakano H, Oussoultzoglou E, Rosso E, Casnedi S, Chenard-Neu MP, Dufour P, Bachellier P, et al. Sinusoidal injury increases morbidity after major hepatectomy in patients with colorectal liver metastases receiving preoperative chemotherapy. Ann Surg 2008;247:118-24.