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Quantitative Assessment of Hepatic Fibrosis by Contrast-enhanced Ultrasonography[△]

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Key words: hepatic fibrosis; contrast-enhanced ultrasonography; quantitative; non-invasive

Objective To explore the contrast-enhanced ultrasonographic features for quantitative assessment of hepatic fibrosis.

Methods 86 patients with chronic viral hepatitis B were enrolled in this study from March 2007 to August 2009. The patients were classified into 5 groups (S_0 - S_4) according to fibrosis stage evaluated with ultrasound guided liver biopsy. New contrast-enhanced ultrasonography (CEUS) features including area under the time-intensity curve (TIC) of portal venous phase/hepatic arterial phase (Qp/Qa) and intensity of portal venous phase/hepatic arterial phase (Ip/Ia) were used to detect the blood supply ratio (portal vein/hepatic artery) in each group. Arrival time of portal vein trunk (Tp) and decreasing rate of TIC (β) were also analyzed.

Results Qp/Qa and Ip/Ia decreased from S_0 to S_4 , while Tp and β increased. These 4 features were significantly correlated with the degree of fibrosis ($P < 0.001$) and were significantly different among the five groups ($P < 0.001$). Sensitivity and specificity of Ip/Ia were 80% and 86% for groups $\geq S_1$, 75% and 86% for groups $\geq S_2$, 71% and 84% for groups $\geq S_3$, and 76% and 80% for group S_4 , respectively. Sensitivity and specificity of Qp/Qa were 70% and 88% for groups $\geq S_1$, 80% and 76% for groups $\geq S_2$, 74% and 70% for groups $\geq S_3$, and 81% and 95% for group S_4 , respectively.

Conclusion Ip/Ia and Qp/Qa could be adopted as reliable, non-invasive features for quantitative assessment of hepatic fibrosis.

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HEPATIC fibrosis is a wound-healing response to chronic hepatopathy. It may develop to liver cirrhosis, which has severe complications including portal hypertension, ascites, encephalopathy,

and impaired metabolic capacity.¹ In patients with chronic hepatopathy, the fibrosis degree is an important factor as it helps to decide therapeutic options and predict prognosis.²

Liver biopsy is the current gold standard for the diagnosis of hepatic fibrosis. However, this procedure is highly invasive with inevitable problems of morbidity and sampling errors.^{3,4} In addition, repeating liver biopsies are not well tolerated, thus not appropriate for monitoring

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disease progression or response to anti-fibrosis therapies. Therefore, there is an urgent need for a non-invasive and more reliable method to evaluate hepatic fibrosis quantitatively.

Based on hepatic perfusion imaging, researches on such non-invasive assessment of hepatic fibrosis have been conducted in computed tomography (CT), magnetic resonance (MR), and nuclear medicine. Most of them fail to provide a reliable method,^{5,6} since the temporal resolution of these modalities is too low to evaluate real-time enhancement accurately. Additionally, high cost, being time-consuming, and radiation hazard restrict their applications in routine clinical practice. The real-time ultrasound imaging, an inexpensive technique that is repeatable and free from ionizing radiation, has been used for assessment of a variety of hepatic diseases. Contrast-enhanced ultrasonography (CEUS) is a new ultrasound technique which can provide hemodynamic information of blood circulation and tissue blood flow. A variety of CEUS features, such as arrival time of hepatic vein, transit time of hepatic vein, and delayed time of carotid artery, have been investigated to assess hepatic diseases.⁷⁻¹⁰ The results show that these CEUS features may be useful for diagnosis of liver cirrhosis, but have no clear diagnostic value for mild and moderate hepatic fibrosis. Elastasonography may be helpful for assessing hepatic fibrosis, but is still under investigation.¹¹

Under the condition of low mechanical index, microbubble-based ultrasound contrast agent, like the other pure blood pool agents, can stay stable for a period of time, neither discharged from capillaries nor diffused into interstitium. According to tracer dilution principle in imaging, within a certain concentration, the signal intensity of CEUS is related to the concentration of the agent, also to blood flow of tissue.¹² Thus, the time-intensity curve (TIC) of CEUS can quantitatively assess blood flow of tissue microcirculation.¹³ This technique has been applied to clinical evaluation of real-time myocardial perfusion.¹⁴ Theoretically, it can also be used to evaluate real-time blood flow of liver.

In this study, we presumed that the pathological changes of hepatic fibrosis would be accompanied by changes in the blood supply ratio (portal vein / hepatic artery) in the hepatic microcirculation. Therefore, CEUS features derived from hepatic parenchyma TIC may help quantitative evaluation of hepatic fibrosis.

PATIENTS AND METHODS

Patients

This study was approved by the Ethics Committee of Peking University Third Hospital. Before the study, the written informed consents were obtained from all the patients. From March 2007 to August 2009, 106 patients with chronic viral hepatitis B were initially included into the present study. Among them, 5 patients were of chronic kidney disease, 11 patients had surgery or intervention procedure of the portal vein, and 4 patients were of portal venous thrombus; thus those 20 cases were excluded. Eventually 86 patients were enrolled in this study, including 48 males and 38 females with a mean age of 45.2 ± 7.6 years (range, 30-58 years).

Liver biopsy and hepatic fibrosis stage

The enrolled patients underwent an ultrasound guided liver biopsy with 18 gauge needle (BARD Magnum, BARD Inc, Tempe, AZ, USA). Three core specimens were obtained from each patient, which were evaluated by two experienced pathologists. All the liver biopsies were performed within 1 week before or after CEUS examinations.

According to the Metavir scoring system, hepatic fibrosis was staged on a scale from 0 to 4 as follows: S_0 , no fibrosis; S_1 , portal fibrosis without septa; S_2 , portal fibrosis and few septa; S_3 , numerous septa without cirrhosis; and S_4 , early cirrhosis.¹⁵ Based on the biopsy findings and staging category, the 86 patients were classified into 5 groups: S_0 ($n=10$), S_1 ($n=10$), S_2 ($n=22$), S_3 ($n=9$), and S_4 ($n=35$).

Ultrasound contrast agent

SonoVue, microbubbles of SF₆ coated by phospholipids (Bracco Diagnostic Inc, Milan, Italy), was used in this study. The mean diameter of it was 2.5 μ m, the pH was within the range of 4.5-7.5. Using 5 mL normal saline, the suspension of the agent was produced by vigorous shaking for 10 seconds.

Instrument and CEUS technique

Hepatic CEUS examinations were performed using the ultrasound diagnosis system with broad-band frequency (2-5MHz) convex array probe (iU22, Philips HealthCare, Royal Philips Electronics Inc, Amsterdam, Netherlands). Real-time harmonic imaging of CEUS with a mechanical index of 0.06 was utilized in this study. Tissue gain and contrast gain were adjusted to 80%. The depth was controlled to 14 cm. All the setup parameters remained the same in all the patients.

Two sections were selected to acquire CEUS images: the liver-kidney section and the right liver section. For the liver-kidney section (Fig. 1), 1.0 mL SonoVue suspension

was injected through an elbow vein followed by a bolus injection of 5 mL normal saline. Timer on the ultrasound system was started to record the time before the agent arriving at the kidney, and the patients were required to hold their breath as long as possible. A dynamic ultrasound imaging from contrast enhancement to reduction was recorded digitally on the hard drive in the ultrasound system. For the right liver section (Fig. 2), on the other hand, additional 2.5 mL SonoVue was administered using the same technique 10 minutes after the first injection. Real-time contrast imaging of the right liver was obtained and stored for later measurement and analysis.

Generation of TICs

TICs were generated from CEUS imaging clips on a PC-based workstation using SonoLiver 1.0 software (Image-Arena workstation, TomTec Inc, Munich, Germany). For the liver-kidney section, a part of the hepatic parenchyma and a part of the renal cortex were chosen as the regions of interest (ROI) to generate the TICs simultaneously (Fig. 1). For the right liver section, a part of hepatic parenchyma was chosen as the ROI to generate the TIC for assessment (Fig. 2). Quality of fit of the TICs was over 75%.

All the TICs were analyzed using SonoLiver 1.0 software by two experienced ultrasound physicians who were blind to the patient information and pathologic findings.

Identification of CEUS features

CEUS images of the right liver section were replayed and reviewed in a frame by frame fashion. Portal vein trunk was closely observed. The time when a cluster of microbubbles first appeared in the portal vein trunk was considered as arrival time of portal vein trunk (T_p).

The liver-kidney section was the assistant section for identification of different phases. In this section, the time when a renal intensity reached the peak was used as the demarcation between hepatic arterial phase and portal venous phase. The right liver section was the detection section used for generation of CEUS features. Demarcation time on the liver-kidney imaging section was transferred onto the right liver section as T_a . So in the right liver section, the hepatic arterial phase was defined as the duration from the arrival time of liver parenchyma to T_a , and the portal venous phase was defined as the duration from T_a to the peak time of liver parenchyma. Other CEUS features on the right liver section were defined as follows:

Ia: intensity at the time of T_a ; Ipeak: maximum intensity of TIC; Tpeak: the time when TIC arrived at Ipeak; Ip: Ipeak-Ia, representing the continuously increasing

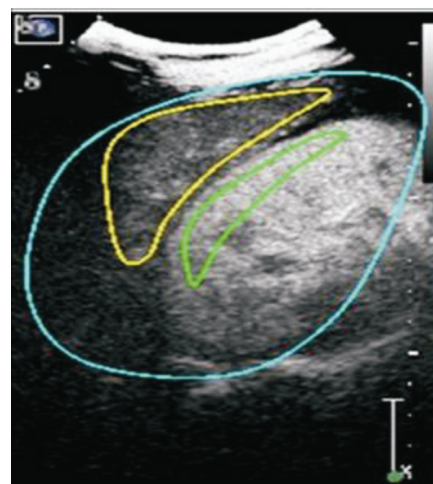


Figure 1. Contrast-enhanced ultrasonography (CEUS) image of the liver-kidney section. The region marked by yellow is the region of interest (ROI) of the hepatic parenchyma and the green line circles the ROI of the renal cortex. Both selected regions must be within the blue circle, as required by the software for choosing ROIs.

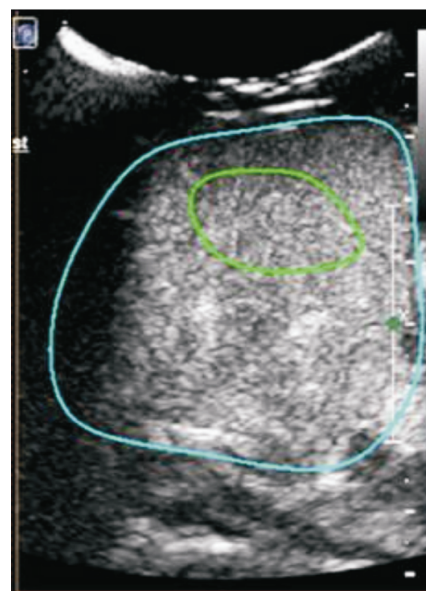


Figure 2. CEUS image of the right liver section (the probe placed on intercostal mid-axillary line). The region inside the green circle stands for the ROI of hepatic parenchyma.

intensity during the portal venous phase; β : decreasing rate of TIC (calculated by SonoLiver 1.0 software); Q_p/Q_a : area under curve (AUC) of portal venous phase/hepatic arterial phase = $\int_{T_a}^{T_{peak}} I(t)dt / \int_0^{T_a} I(t)dt$; I_p/I_a : intensity of portal venous phase/hepatic arterial phase.

Receiver operating characteristic (ROC) curves were generated, AUCs were analyzed to evaluate the diagnostic accuracy of Q_p/Q_a and I_p/I_a , and determine demarcation values for each group, then sensitivity and specificity were calculated for each value.

Statistical analysis

Statistical software SPSS15.0 was used for data analysis. Differences among groups were analyzed by analysis of variance and the inter-group comparisons were analyzed by Fisher's least significant different post hoc test. Spearman rank correlation test was applied to analyze correlations between features and the degree of fibrosis. $P < 0.05$ was considered statistically significant.

RESULTS

The comparison between TICs of normal (S_0) and fibrosis (S_3) liver on CEUS was presented in Figures 3 and 4 as an example. The duration of hepatic arterial phase was shorter than that of portal venous phase (Fig. 3) in normal liver. But in fibrosis liver, the duration of hepatic arterial phase increased while the duration of portal venous phase decreased (Fig. 4), and I_p/I_a and Q_p/Q_a declined while T_p and β were increased. Features in different groups indicated that the averages of T_p and β increased while those of I_p/I_a and Q_p/Q_a decreased from S_0 to S_4 (Table 1).

The comparisons between CEUS features and fibrosis groups showed that T_p , β , I_p/I_a , and Q_p/Q_a were significantly correlated with the degree of fibrosis ($P < 0.001$, Table 2).

Further evaluation showed that all the features (T_p , β , I_p/I_a , and Q_p/Q_a) were significantly different among the five groups ($P < 0.001$, Table 3). Inter-group comparisons showed some differences as follows: T_p could identify S_4 but failed to make distinction among S_0 - S_3 ; β could separate more groups apart than T_p did, but failed to differentiate adjacent groups such as S_0 vs. S_1 , S_1 vs. S_2 , and S_2 vs. S_3 . It was noticed that I_p/I_a and Q_p/Q_a were more favorable features for separating all the stages apart, except for S_2 vs. S_3 .

The corresponding area under receiver operating characteristic (AUROC) curves for I_p/I_a were 0.931 for groups $\geq S_1$, 0.884 for groups $\geq S_2$, 0.820 for groups $\geq S_3$ and 0.846 for group S_4 , respectively. And that for Q_p/Q_a were 0.869 for groups $\geq S_1$, 0.845 for groups $\geq S_2$, 0.813 for groups $\geq S_3$, and 0.914 for group S_4 , respectively (Table 4, Figs. 5, 6).

The sensitivity and the specificity of I_p/I_a and Q_p/Q_a were determined for each fibrosis group (Table 5). The sensitivity and the specificity of I_p/I_a were 80% and 86% for groups $\geq S_1$, 75% and 86% for groups $\geq S_2$, 71% and 84% for groups $\geq S_3$, and 76% and 80% for group S_4 , respectively. Q_p/Q_a was better at identifying group S_4 with a sensitivity of 81% and a specificity of 95%.

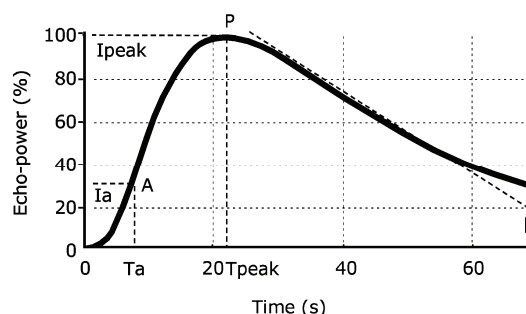


Figure 3. Time-intensity curve (TIC) of normal hepatic parenchyma on CEUS. Point A is the demarcation of hepatic arterial phase and portal venous phase. Point P is the peak of TIC. T_a and I_a stand for the time and the intensity of point A. The area under curve (AUC) of portal venous time and that of hepatic arterial time are expressed as Q_p and Q_a respectively. T_{peak} stands for the time of point P, and I_{peak} for the intensity of point P. The arrival time of portal vein trunk is expressed as T_p . β is the decreasing rate of TIC.

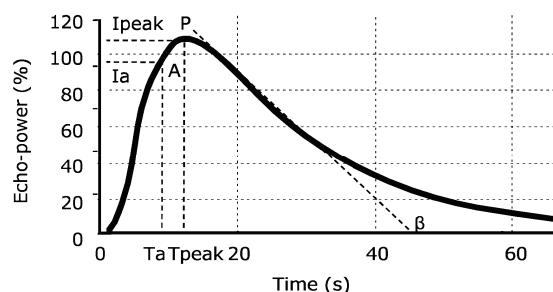


Figure 4. TIC of fibrosis hepatic (S_3) parenchyma on CEUS. The hepatic arterial time ratio increases while the portal venous time ratio decreases. I_{peak}/I_a and Q_p/Q_a were reduced while T_p and the β were elevated.

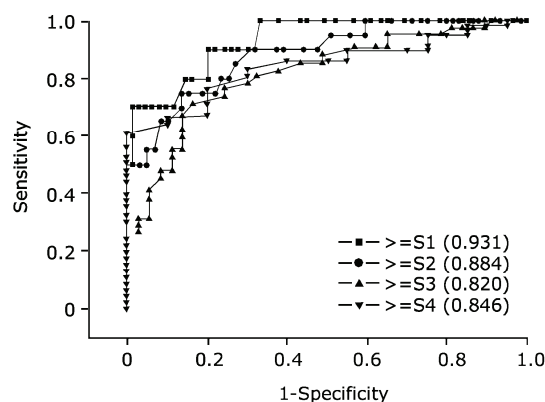


Figure 5. Receiver operating characteristic (ROC) curves of I_p/I_a from S_1 to S_4 . The area under receiver operating characteristic (AUROC) curves were 0.931 for groups $\geq S_1$, 0.884 for groups $\geq S_2$, 0.820 for groups $\geq S_3$, and 0.846 for group S_4 .

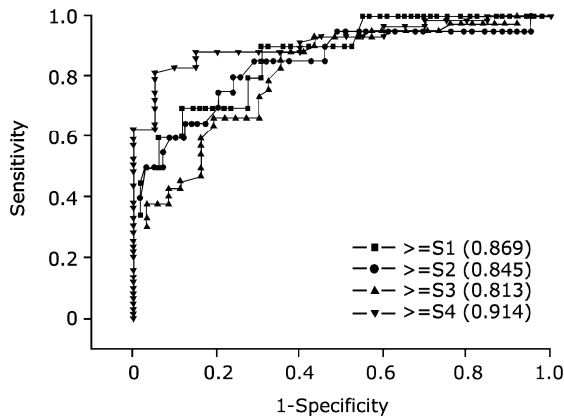


Figure 6. ROC curves of Qp/Qa from S_1 to S_4 . The AUROC curves were 0.869 for groups $\geq S_1$, 0.845 for groups $\geq S_2$, 0.813 for groups $\geq S_3$, and 0.914 for group S_4 .

Table 1. CEUS features in different groups of hepatic fibrosis[§]

Groups	Features			
	Tp (s)	β	Ip/Ia	Qp/Qa
S_0 ($n=10$)	3.89 ± 1.26	14.8 ± 5.6	1.34 ± 0.31	9.02 ± 2.32
S_1 ($n=10$)	4.91 ± 2.06	16.5 ± 3.1	1.00 ± 0.22	7.37 ± 2.12
S_2 ($n=22$)	4.89 ± 1.82	19.7 ± 3.0	0.83 ± 0.18	6.16 ± 1.37
S_3 ($n=9$)	5.28 ± 2.00	20.0 ± 4.0	0.76 ± 0.20	5.82 ± 1.33
S_4 ($n=35$)	7.72 ± 2.22	23.4 ± 5.6	0.61 ± 0.13	4.00 ± 0.89

§: Plus-minus values are means $\pm$ SD.

Table 2. Correlation between CEUS features and the degree of fibrosis

Correlation	Tp (s)	β	Ip/Ia	Qp/Qa
Correlation coefficient	0.498	0.486	-0.686	-0.708
P	<0.001	<0.001	<0.001	<0.001

Table 3. P value for comparisons of CEUS features

Comparisons	Tp (s)	β	Ip/Ia	Qp/Qa
S_0 - S_4	<0.001	<0.001	<0.001	<0.001
S_0 vs. S_1	0.245	0.402	<0.001	0.018
S_0 vs. S_2	0.181	0.004	<0.001	<0.001
S_0 vs. S_3	0.077	0.004	<0.001	<0.001
S_0 vs. S_4	<0.001	<0.001	<0.001	<0.001
S_1 vs. S_2	0.980	0.053	0.027	0.041
S_1 vs. S_3	0.635	0.044	0.003	0.013
S_1 vs. S_4	<0.001	<0.001	<0.001	<0.001
S_2 vs. S_3	0.538	0.837	0.287	0.495
S_2 vs. S_4	<0.001	0.009	0.001	<0.001
S_3 vs. S_4	<0.001	0.023	0.025	<0.001

Table 4. AUROC curves for CEUS features in different groups

Groups	Tp (s)	β	Ip/Ia	Qp/Qa
$\geq S_1$	0.240	0.236	0.931	0.914
$\geq S_2$	0.304	0.207	0.884	0.813
$\geq S_3$	0.269	0.294	0.820	0.845
S_4	0.169	0.264	0.846	0.869

Table 5. Sensitivity and specificity of Ip/Ia and Qp/Qa with cutoff values in different groups

Groups	Ip/Ia			Qp/Qa		
	Cutoff value	Sensitivity	Specificity	Cutoff value	Sensitivity	Specificity
$\geq S_1$	1.02	0.80	0.86	7.70	0.70	0.88
$\geq S_2$	0.96	0.75	0.86	6.50	0.80	0.76
$\geq S_3$	0.83	0.71	0.84	5.60	0.74	0.70
S_4	0.72	0.76	0.80	5.05	0.81	0.95

DISCUSSION

After intravenous injection of the agent, with lower acoustic incident power, the echo intensity is directly proportional to the scattering cross section of the imaging, according to CEUS theory. Thus, with certain incident frequency of ultrasound beam and scatter radius of the microbubbles, the intensity of scattering cross section is directly proportional to the amount of scatters within the imaging plane. Therefore, CEUS echo intensity is related to the concentration of microbubbles and also related to blood flow of tissue, based on tracer dilution principle.¹²

Ip/Ia and Qp/Qa proposed in this study are new CEUS features for evaluating blood flow ratio of portal vein/hepatic artery. Their advantages over absolute values, such as peak intensity, are that both features are obtained from the same TIC derived from the same ROI. The ratio could therefore eliminate the influence of many variables such as position of the probe, body shape of the patients, concentration of the agent, depth, area and shape of the ROI, and instrument settings, etc.

The right liver and the liver-kidney sections were used in this study. The former one was detection section for generation of CEUS features while the latter one was assistant section used for identification of hepatic arterial phase and portal venous phase. It has to be noticed that the time point on TIC could not separate the blood supply of hepatic artery and portal vein completely. The deliberate identification of the two phases in this research was used to generate features that were most appropriately representative of the proportional change of portal vein and hepatic artery.

The time when renal intensity reached the peak in the liver-kidney section was used as the demarcation of hepatic arterial phase and portal venous phase in the TIC of the right liver section, because both sections showed part of the same liver. Based on an assumption that the two parts began to enhance at the same time, the arrival time

of liver was marked as zero point. The idea of making the peak time of renal cortex as demarcation of portal vein and hepatic artery was adapted from CT. In hepatic perfusion imaging of CT, researchers used the peak time of spleen and renal cortex in time density curve to make a distinction between hepatic arterial phase and portal venous phase.¹⁶ Since the spleen was hardly to be showed within the same section as the liver, the kidney was used as a reference for demarcation.

The hepatic blood supply is provided by both portal vein and hepatic artery. In the course of hepatic fibrosis, various factors lead to continuously deposit of collagen fibers in the Disse space. The deposition of collagen fibers causes damage of liver sinusoidal endothelial cells and leads to micro-thrombosis.¹⁷ All the above factors lead to increasing of intra-hepatic vascular resistance. Combined with intra-hepatic shunt,¹⁸ the pathological changes reduced blood flow proportion of portal vein. Meanwhile, the hepatic artery, due to high pressure, is less affected by increasing resistance. In addition, the hepatic arterial buffer response provides compensatory increasing blood flow, which enhances blood flow proportion of hepatic artery.¹⁹ Thus, in the course of hepatic fibrosis, blood flow ratio of portal vein/hepatic artery would decrease, which was confirmed in the present study. Findings of declining I_p/I_a and Q_p/Q_a in this study are consistent with the theory of histopathologic and hemodynamic changes of hepatic fibrosis mentioned above.

Based on that theory, researchers tried to use CT perfusion imaging to detect hepatic arterial and portal venous blood flow in different fibrosis groups. Result showed an increasing hepatic arterial blood flow and a decreasing portal venous blood flow as the fibrosis became more severe.²⁰ Ronot et al²¹ used perfusion CT to discriminate minimal fibrosis (F_1) from intermediate fibrosis (F_2 and F_3). The results showed that mean transit time appeared to be the most promising perfusion parameter for differentiating between fibrosis stages, and that a mean transit time threshold of 13.4 seconds allowed discrimination between minimal and intermediate fibrosis with a sensitivity of 71% and a specificity of 65%. In addition, the distribution volume parameter demonstrated a sensitivity of 77% and a specificity of 79% for the diagnosis of advanced fibrosis.²²

Compared with ultrasound, CT can provide higher quality and whole liver image, and multidetector CT scanner has a largely enhanced scanning speed. However, CT perfusion imaging has its limitations of radiation and the use of iodinated contrast agents. MR imaging may be a promising alternative to perfusion CT, but result of

Hagiwara showed that magnetization transfer contrast is not a specific indicator of increased fibrosis in diseased liver; steatosis may influence some perfusion parameters.²³

CEUS has high time resolution (about 20 frames/second), which contributes to assessing hemodynamic changes of hepatic fibrosis (blood supply proportion of hepatic artery and portal vein). Furthermore, CEUS is free of radiation and renal toxicity, thus is safer than CT and less expensive than MR.

Elastosonography is also a non-invasive method to assess hepatic fibrosis by measuring liver stiffness. The transient elastography (TE) and the acoustic radiation force impulse imaging (ARFI) are two commonly used methods of elastosonography. Timo et al²⁴ studied 55 patients with chronic liver disease, and the results showed that the AUROC of TE were 0.798, 0.880, and 1 for $F \geq 2$, $F \geq 3$, and $F = 4$, respectively (significant fibrosis, $F = 2$; severe hepatic fibrosis, $F = 3$; cirrhosis, $F = 4$). Lupsor et al²⁵ studied 112 chronic hepatitis C patients using both ARFI and TE, and the adjusted AUROC according to fibrosis stage for ARFI vs. TE was: 0.709 vs. 0.902, $P = 0.006$ (for $\geq F_1$); 0.851 vs. 0.941, $P = 0.022$ (for $\geq F_2$); 0.869 vs. 0.926, $P = 0.153$ (for $\geq F_3$); 0.911 vs. 0.945, $P = 0.331$ (for F_4). Most studies show that elastography measurements are more accurate in diagnosing severe fibrosis and cirrhosis than less or moderate fibrosis.^{26, 27} Further studies are still needed to make comparison between elastography and the CEUS features in our study.

In the course of hepatic fibrosis, hepatic artery/hepatic vein and portal vein/hepatic vein shunts reduce hepatic blood flow, aggravated by increasing of intra-hepatic vascular resistance such as narrowing sinusoid, thus resulting in fast decreasing hepatic blood flow, which is confirmed by the increasing β in this study. Meanwhile, increasing intra-hepatic vascular resistance induces longer portal venous time, consistent with the change of T_p in this study. Although β and T_p showed less diagnostic value in this study, they could be supplementary to I_p/I_a and Q_p/Q_a for assessment of hepatic fibrosis.

There are some limitations in this research: (1) motion artifacts of CEUS images, which affected the accuracy of TICs; (2) sample errors of liver puncture biopsy; (3) lack of comparison between CEUS and other imaging modalities; (4) limited number of patients enrolled.

In conclusion, I_p/I_a and Q_p/Q_a are favorable predictors for the diagnosis of hepatic fibrosis while β and T_p have moderate diagnostic value. CEUS with measurements of hepatic blood supply features have the potential to be applied as a non-invasive method for

evaluating the severity of hepatic fibrosis. Further investigations with large samples are needed to confirm the findings.

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