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Effect of Hepatic Inflammation in Chronic Hepatitis C Infection on Fibrosis Assessment by Arrival Time Parametric Imaging

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Abstract: Arrival time parametric imaging (At-PI) in contrast-enhanced ultrasonography is useful for assessing liver fibrosis in chronic hepatitis C (CHC) infection. The study aimed to elucidate the effect of hepatic inflammation on At-PI efficiency. Subjects were 159 CHC patients who underwent contrast-enhanced ultrasonography immediately before liver biopsy. Ultrasound contrast agent was injected, and contrast dynamics of the S5 to S6 region of the liver and right kidney were recorded for 40 seconds. The At-PI of liver parenchyma blood flow was generated using saved video clips. Hepatic blood flow during the first 5 seconds after starting contrast injection was displayed in red and that after another 5 seconds was displayed in yellow. The ratio of red (ROR) in At-PI images of the entire liver was measured with ImageJ. Ratio of red values of livers with different activity grades (0–3) were compared for each fibrosis (F) stage as determined by biopsy. Correlations of ROR with alanine aminotransferase (ALT) levels were analyzed using a linear regression line from the distribution map. Comparison of ROR for different activity grades in each F stage revealed no significant differences. Correlation coefficient R (P value) for ALT and ROR was $R = -0.0094$ ($P = 0.43$) at F0 to F1, $R = -0.186$ ($P = 0.21$) at F2, $R = -0.233$ ($P = 0.27$) at F3, and $R = 0.041$ ($P = 0.89$) at F4, with no significant correlation between ALT and ROR in any F stage. Hepatic inflammation in CHC infection does not affect At-PI diagnostic accuracy.

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The authors declare no conflict of interest.

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Liver biopsy is the criterion standard for the diagnosis of fibrosis in patients with chronic hepatitis C (CHC) infection. However, the procedure is invasive and associated with complications such as bleeding. It is also disadvantageous in that only 1 local site in the liver can be evaluated at any 1 time. As a noninvasive alternative to liver biopsy, ultrasonography (US)-based techniques that visualize and measure liver stiffness have been developed in recent years, and the efficacy of these techniques in diagnosing liver fibrosis has been reported.[1–5] Nevertheless, hepatic inflammation is known to affect the efficacy of these techniques and results in the overestimation of the fibrosis stage.[6–9]

In our previous study, we developed a novel contrast-enhanced US technique to quantitatively assess the change in hepatic blood flow from portal venous dominant to arterial dominant, and we reported the efficacy of the technique for assessing liver fibrosis in patients with CHC infection.[10] However, it was not clear whether fibrosis assessment was affected by the severity of the infection. Therefore, in this study, we evaluated the influence of inflammation on contrast-enhanced US for CHC infection.

MATERIALS AND METHODS

Patients

We recruited 167 patients with CHC infection for 7 years. All evaluations were conducted at the Department of Gastroenterology, Toho University Hospital Omori Medical Center, Japan. Before interferon therapy, liver biopsy was performed to evaluate the pathological state of the liver. Chronic hepatitis C was diagnosed based on a positive result in HCV-RNA quantitation by TaqManPCR (Invitrogen, Carlsbad, Calif) and the absence of HBsAg and HBcAb. Patients with a daily alcohol consumption of more than 80 g, heart and kidney disease, significant portal collaterals, hepatic tumor, or portal vein thrombosis were excluded. Patients with poor imaging

of the liver due to, for example, a narrow intercostal space, were also excluded.

After excluding 8 patients (narrow intercostal spaces, n = 5; inability to hold breath, n = 3), 159 patients comprising 92 men and 67 women aged 55 ± 11 (range, 21–85) years were included in the analysis. The study protocol was in accordance with the Declaration of Helsinki and was approved by the ethics committee of our institution (no 26–227). Written informed consent was obtained from all participants.

Contrast-Enhanced Ultrasonography

Ultrasonography was performed from the right intercostal space using a Canon AplioXG (SSA-790A; Canon Medical Systems, Tochigi, Japan) with a 3.75-MHz convex array probe (PVT-375BT). The mechanical index (MI) and frame rate were set to 0.22 to 0.29 and 15 to 18 frames per second, respectively. Images showing liver parenchyma of the right hepatic lobe (segment 5 or 6) and the right kidney were used in analysis. Focus was set to 6 to 8 cm to cover the whole kidney. Participants were examined in the supine position with the right arm elevated above the head and instructed to hold their breath. All patients were fasted overnight before the examination. After setting imaging parameters, the recommended dose (0.015 mL/kg) of the second-generation contrast agent Sonazoid for contrast-enhanced US[11] (perfluorobutane; GE Healthcare, Oslo, Norway) was administered as a bolus via the cubital vein at a rate of 1 mL/s and flushed with 10 mL of normal saline. The start of the cine acquisition is beginning of saline flush. Data generated for the first 40 seconds were saved as raw data in the system hardware. All ultrasound examinations were performed by an independent examiner with over 24 years' experience as an ultrasonographer who was blinded to patient characteristics.

Arrival Time Parametric Imaging

The software interfaced with the ultrasound system was used to generate arrival time parametric imaging (At-PI) images from stored video clips. By simply selecting the renal parenchyma as the region of interest (ROI), the system set the point at which 80% of the ROI was contrasted as time 0 and sequentially calculated arrival time in individual pixels of the hepatic parenchyma. The system then automatically created and superimposed a color map on a B-mode image. The difference in the arrival times of arterial and subsequent portal venous blood to the liver was reported to be 5 seconds.[12] From the freely selectable display colors, we therefore used red and yellow to display pixels arriving at 0 to <5 seconds and at ≥5 to 10 seconds, respectively. In other words, red and yellow indicate the liver parenchyma nourished by blood through the arterial and venous route, respectively (Fig. 1).

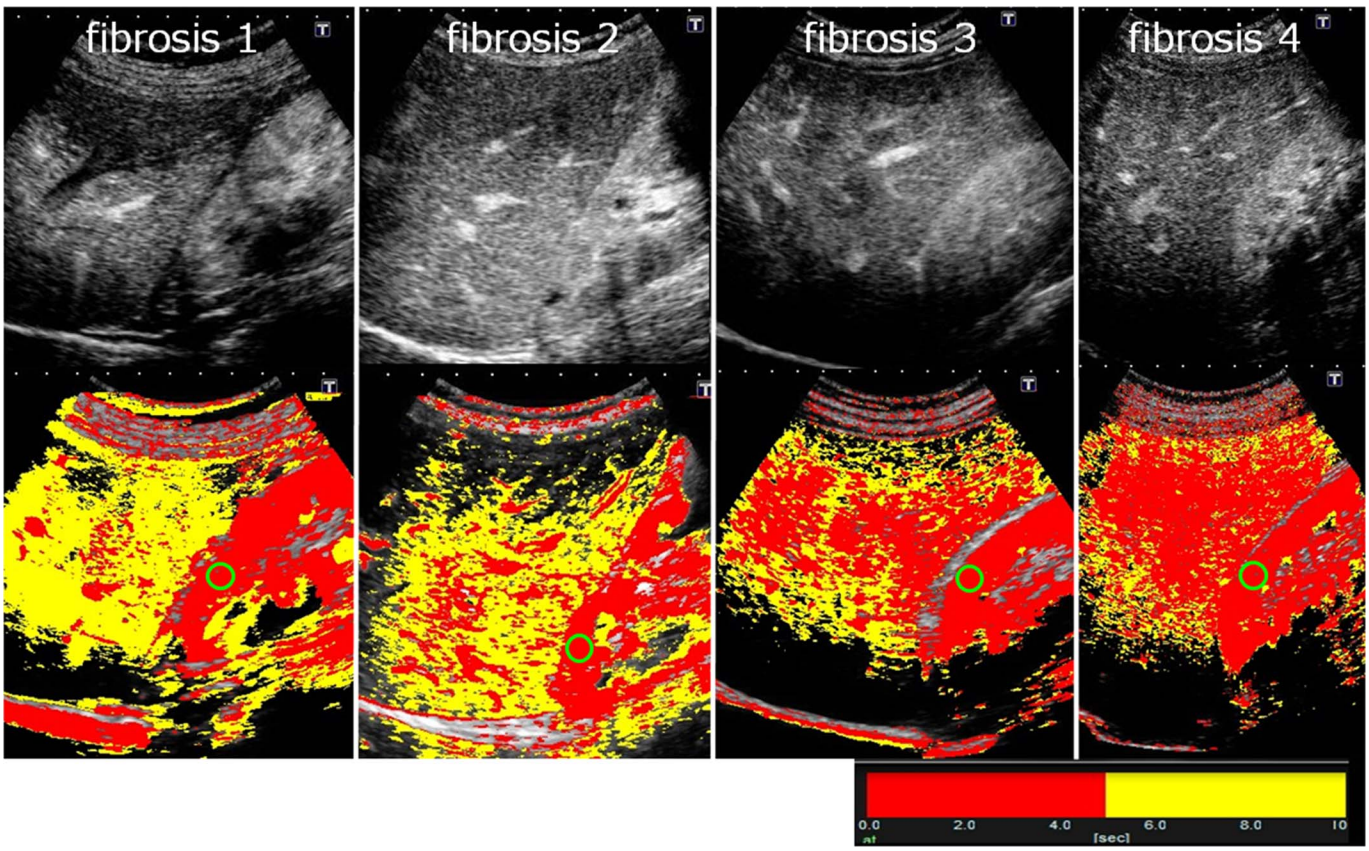

FIGURE 1. The upper and lower panels show B-mode and At-PI images, respectively. These images were obtained from patients with fibrosis stages F1, F2, F3, and F4 (left to right). By simply selecting the renal parenchyma as the ROI, the system set the point at which 80% of the ROI was contrasted as time 0 and sequentially calculated arrival time in individual pixels of the hepatic parenchyma. We used red and yellow to display pixels arriving at 0 to <5 seconds and at ≥5 to 10 seconds, respectively.

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TABLE 1. Clinical and Biochemical Characteristics of the Patients

Characteristics	Chronic Liver Disease Patients (N = 159)
Sex, male/female	92/67
Age, y	55 ± 11
Alanine aminotransferase, IU/L	67.3 ± 49.7
Platelet count, × 10^4/μL	14.6 ± 6.0
Total bilirubin, mg/dL	0.8 ± 0.3
Prothrombin time, % of normal	92.8 ± 13.8
Albumin, g/dL	4.0 ± 0.4
Total cholesterol, mg/dL	161.4 ± 26.4
Fibrosis stage	
F0 to F1	72
F2	47
F3	25
F4	15
Activity score	
A0	3
A1	67
A2	89
A3	0

Values are expressed as means ± standard deviation.

Measurement of Red Area

For quantitative evaluation of the obtained At-PI data, the ratio of the area of red pixels with shorter arrival times to the entire contrast-enhanced area was calculated as the "ratio of red" (ROR) in ImageJ version 1.42 image analysis software[10] (Wayne Rasband, National Institutes of Health, Bethesda, Md). The regions where arrival of the contrast agent was detected within 5 seconds were depicted in red to calculate the ROR. A higher ratio indicates that the contrast agent arrival time in the liver is closer to that in the kidney. In other words, a wider area of the liver parenchyma received the contrast agent through the arterial route, indicating a shift in the arterial-portal blood flow balance toward arterial domination in the liver.

To calculate the ROR, ImageJ was used to select and measure only the areas in red in the liver parenchyma on arrival time parametric images. Next, the entire area of contrast enhancement in the liver parenchyma was displayed in the same color to measure the area. Lastly, the calculation of ROR was performed by 2 physicians. Both were physicians trained in the use and interpretation of contrast agents in the liver. They were not involved in sonographic scanning and were blinded to the identification, clinical history, and other imaging findings of the patients. Both physicians analyzed the ROR together. Using ImageJ software, 1 physician measured the ratio, and the other examined each case and evaluated the accuracy of the ratio measurement performed by the other physician.

Correlation Between Hepatic Inflammation and ROR in Each Fibrosis Stage

Liver needle biopsy was performed after sonography with a 16-gauge liver biopsy needle (Core II semiautomatic biopsy instrument; InterV Clinical Products, Dartmouth, Mass), and the specimen was obtained from the anterior segment of the right lobe under US guidance. The specimen was fixed in 10% formalin, embedded in paraffin, sectioned, and stained with hematoxylin-eosin and azan for histological evaluation. All liver biopsy specimens were evaluated by a single experienced pathologist who was unaware of the patient's clinical condition. Pathologic liver fibrosis and inflammation were evaluated according to the Metavir scoring system.[13] Fibrosis was staged as follows: F0, no fibrosis; F1, portal fibrosis without septa; F2, portal fibrosis and few septa; F3, numerous septa without cirrhosis; and F4, cirrhosis. Activity, which is the amount of inflammation, was graded on a 4-point scale from A0 to A3 (A0, no activity; A1, mild activity; A2, moderate activity; A3, severe activity). The hepatic proinflammatory indices consisted of the A grades, and the alanine aminotransferase (ALT) level measured by hemanalysis. In all patients, blood was collected on the day before liver biopsy. The effect of inflammation on ROR was investigated by comparing RORs in different fibrosis (F) stages.

Statistical Analysis

Statistical analysis was performed in Microsoft Excel 2012 (Microsoft Corp, Redmond, Calif), with significance set at 0.05. A comparative analysis of ROR and A grade in each F stage was performed using the Steel-Dwass test. Statistical analysis was also performed to measure the correlation between ALT levels and ROR by measuring the regression coefficient on the distribution map.

RESULTS

Clinical and biochemical characteristics of the patients (N = 159) are summarized in Table 1. The distribution of F stage was as follows: 72 patients had F0 to F1, 47 had F2, 25 had F3, and 15 had F4. Ratio of red in each F stage was 18.9% ± 10.7% for F0 to F1, 31.7% ± 18.4% for F2, 62.6% ± 12.6% for F3, and 82.9% ± 10.8% for F4. The number of patients with different A grades in each F stage was as follows (ROR shown in parentheses): 3 with A0 (19.8% ± 10.4%), 44 with A1 (19.0% ± 11.0%), 25 with A2 (18.8% ± 11.3%), and 0 with A3 at F0 to F1; 0 with A0, 16 with A1 (35.2% ± 21.6%), 31 with A2 (29.8% ± 16.6%), 0 with A3 at F2; 0 with A0, 4 with A1 (70.0% ± 7.0%), 21 with A2 (61.2% ± 13.2%), and 0 with A3 at F3; and 0 with A0, 3 with A1 (82.0% ± 12.0%), 12 with A2 (83.1% ± 11.0%), and 0 with A3 at F4.

Mean ALT value (range) (IU/L) was 58.0 ± 52.0 (3–284) for F0 to F1, 69.4 ± 49.8 (13–192) for F2, 84.6 ± 43.1 (14–186) for F3, and 76.6 ± 41.1 (12–162) for F4 (Table 2). Comparisons of the ROR values between A grades in each F stage revealed no significant differences: A0 versus A1 ($P = 0.94$), A0 versus A2 ($P = 0.99$), and A1 versus A2 ($P = 0.99$) for F0 to F1; A1 versus A2 ($P = 0.53$) for F2; A1 versus A2 ($P = 0.21$) for F3; and A1

TABLE 2. Degree of Inflammation According to the Fibrosis Stage

	A0	A1	A2	A3	ALT, IU/L	ROR, %
F0 to F1	3	44	25	0	58.0 ± 52.0	18.9 ± 10.7
F2	0	16	31	0	69.4 ± 49.8	31.7 ± 18.4
F3	0	4	21	0	84.6 ± 43.1	62.6 ± 12.6
F4	0	3	12	0	76.6 ± 41.1	82.9 ± 10.8

Values are expressed as means ± standard deviation.
F, fibrosis stage; A, activity grade.

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versus A2 ($P = 1.00$) for F4. The correlation coefficient R (P value) for ALT and ROR in each F stage was $R = -0.009$ ($P = 0.43$) for F0 to F1, $R = -0.186$ ($P = 0.21$) for F2, $R = -0.233$ ($P = 0.27$) for F3, and $R = 0.041$ ($P = 0.89$) for F4, with no significant correlation between ALT and ROR (Figs. 2A–D).

DISCUSSION

Percutaneous liver biopsy is considered the criterion standard for fibrosis assessment in chronic liver diseases including hepatitis C infection.[14,15] However, this invasive procedure is associated with a risk of complications such as bleeding,[16] and only 1 local site of the liver can be evaluated at a given time.[17,18] In recent years, as noninvasive alternatives to liver biopsy, US-based techniques such as transient elastography (Fibroscan), real-time tissue elastography, and acoustic radiation force impulse have been developed and applied for the assessment of liver fibrosis.[19–25] However, even with these techniques, patient condition (eg, ascites) and insufficient US penetration can cause measurement errors.[19,22,25] In addition, recent studies have shown that the above techniques are affected by the inflammatory state of the liver,[6–9] and swelling of hepatocytes, interstitial edema, and infiltrates of inflammatory cells may increase liver stiffness.[26]

In our previous study, we successfully revealed changes in blood flow balance unique to the liver in patients with CHC by using Sonazoid-enhanced US to compare contrast dynamics between the liver and the kidney nourished only by the hepatic artery.[10] As the disease progressed from chronic infection to cirrhosis, the time needed to contrast the liver after visualization was significantly shorter compared with that in healthy controls. This result indicates that the blood flow through the liver parenchyma becomes arterial dominant as the disease progresses toward cirrhosis because the hepatic artery increases the blood flow through the liver to compensate for the reduced blood flow from the hepatic portal vein. Consequently, the hemodynamics in the liver parenchyma start to resemble those in the kidney, and this change may be used as a noninvasive indicator of liver fibrosis. However, in our previous study, it was not clear whether the severity of inflammation would affect the diagnostic accuracy of the new technique developed to assess liver fibrosis. In the present study, we therefore performed a

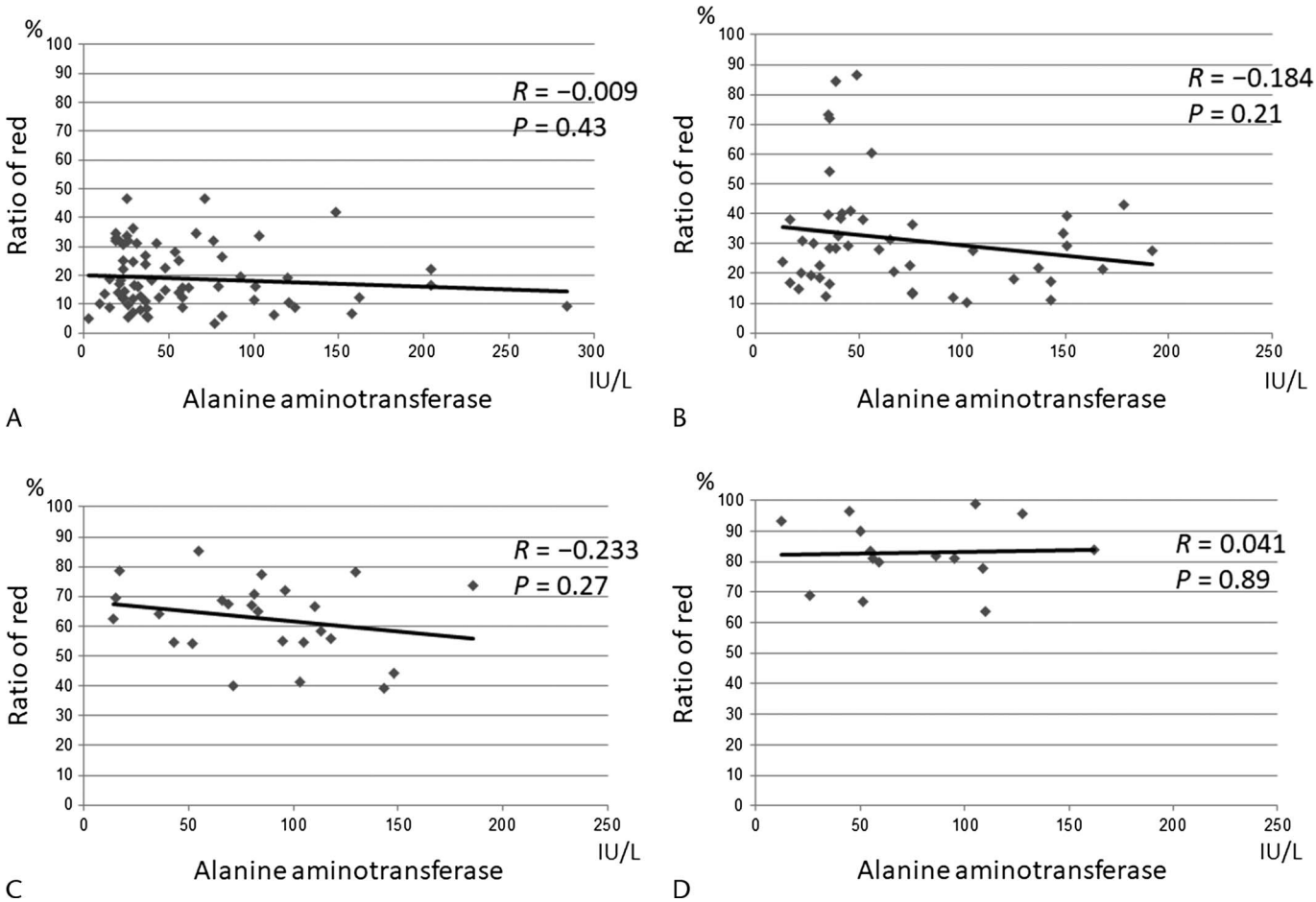

FIGURE 2. Distribution map of A, ROR and ALT values and the linear regression line in F0 to F1 stage (correlation coefficient $R = -0.0094$ [$P = 0.43$] was not significant); B, ROR and ALT values and the linear regression line in F2 stage (correlation coefficient $R = -0.186$ [$P = 0.21$] was not significant); C, ROR and ALT values and the linear regression line in F3 stage (correlation coefficient $R = -0.233$ [$P = 0.27$] was not significant); D, ROR and ALT values and the linear regression line in F4 stage (correlation coefficient $R = 0.041$ [$P = 0.89$] was not significant). X-axis, ALT (IU/L); Y-axis, ROR (%).

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retrospective evaluation to elucidate the effect of inflammation in patients with CHC infection. The results revealed that ROR in each F stage did not vary significantly according to the severity of inflammation, suggesting that hepatic inflammation does not affect At-PI. However, previous a study involving patients with acute hepatic inflammation and ALT > 1000 IU/L reported that the arrival time of Sonazoid in the hepatic and portal veins was altered in the acute and recovery phases of acute liver inflammation.[27]

Multiple factors likely contributed to the present results, which showed that the severity of inflammation does not affect the efficacy of At-PI: no A3 cases were observed in any F stage, and mean ALT was ≤100 in all F stages (58.0 ± 52.0 at F0 to F1, 69.4 ± 49.8 at F2, 84.6 ± 43.1 at F3, and 76.6 ± 41.1 at F4). All patients were administered 600 mg/d of oral ursodeoxycholic acid to suppress the elevation of ALT. It is therefore possible that unlike acute hepatitis, arrival time—the At-PI finding—is not affected in CHC with mild to moderate ALT levels, as seen in this study. The present US technique was used to visualize the right hepatic lobe and right kidney from the right intercostal space and to compare the arrival times of the contrast agent. In other words, this technique is advantageous in that it can provide information about blood flow in the entire right hepatic lobe. This suggests that At-PI findings reflect the severity of disease progression more accurately than liver biopsy, which simply evaluates 1 local site in the liver at a given time.

There were, however, several limitations to this study. The patients had CHC, but the inflammation of the liver was relatively under control. The following diseases and conditions may affect the accuracy of this method: heart diseases associated with possible alterations of the arrival time of the contrast agent to the liver, renal disorders associated with possible alterations of the kinetics of ultrasonic signals in the kidney, heavy drinking habits associated with possible changes in hemodynamics, and portal vein thrombosis associated with possible disturbances of the balance between arterial and portal blood flow. Thus, patients with these diseases or conditions cannot be examined by the present method. Furthermore, patients whose right hepatic lobe cannot be visualized on sonography, such as those with narrow intercostal spaces and those who have difficulty holding their breath for 15 to 20 seconds, must be excluded. In addition, because Sonazoid contains an egg derivative, egg allergy is a contraindication.

The present findings suggest that hepatic inflammation in CHC infection does not affect the outcome of At-PI.

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