manufacturers, but each manufacturer uses its own method for measuring displacement, which results in differences in image characteristics such as spatial and temporal resolution and differences in optimal measurement conditions.

The typical methods for measuring displacement are presented below:

Spatial correlation method (speckle tracking method, pattern matching method)

This is a method that tracks the movement of image patterns. The simplest method is to measure the 1D displacement along the beam axis, as shown in Fig. 7a. Echo signals $s(t)$ and $s'(t)$ before and after compression are clipped out in a time window $w(z; t)$, and their cross-correlation coefficient in Eq. (14) is calculated to evaluate the degree of similarity. Calculation of the cross-correlation coefficient is repeated while moving the window, and the displacement is defined as the amount of movement when the value is at its maximum.

$$R(\delta) = \frac{\int x(z;t)y(z+\delta;t)dt}{\sqrt{\int x^2(z;t)dt \int y^2(z+\delta;t)dt}}, \quad (14)$$

where $x(z; t)$ and $y(z; t)$ are the echo signals clipped out in time window $w(z; t)$ before and after compression, respectively.

In practice, each ROI moves in the azimuthal direction in the cross-section. Therefore, to obtain the displacement more accurately, adjustment is made by performing a two-dimensional (2D) search in both the range direction and the azimuthal direction, as shown in Fig. 7b. In addition, tissue also moves in the slice direction, but the beam width in the slice direction is generally large in the case of an ordinary electronic scanning probe, so the impact is smaller than for the azimuthal direction as long as the cross-section does not deviate much.

Phase difference detection method (Doppler method)

In this method, the phase difference between echo signals obtained by transmitting repeated pulses is detected by an autocorrelation method to calculate the displacement, which is basically the same method as that used in color Doppler and tissue Doppler [21].

The advantages of the Doppler method are its excellent real-time capability and its relative robustness against noise, but only 1D displacement in the beam direction can be measured due to angle dependence, and errors occur due to aliasing when measuring large displacements that exceed half the wavelength.

The advantages of the speckle tracking method are that it is possible to measure even large displacements that exceed the wavelength if the change in the speckle pattern is within a small range and that it is possible to track the movement of the ROI in 2D and three dimensions (3D), as stated above. However, the disadvantage is that the real-time capability may be lost because calculation of correlation requires enormous computational effort. In addition, it is susceptible to the effects of noise, and it is prone to detection errors when the speckle pattern is unclear due to a low echo level, etc.

Combined method

A combined autocorrelation method that combines the merits of the phase difference detection method and the spatial correlation method has been developed [13, 16]. In a clinical case, a wide dynamic range of strain is required, since fluctuations in the speed of compression are large in the case of manual compression. Therefore, a rough displacement is first calculated from the envelope at resolution of half a wavelength, then the displacement is calculated at high resolution using the phase difference by

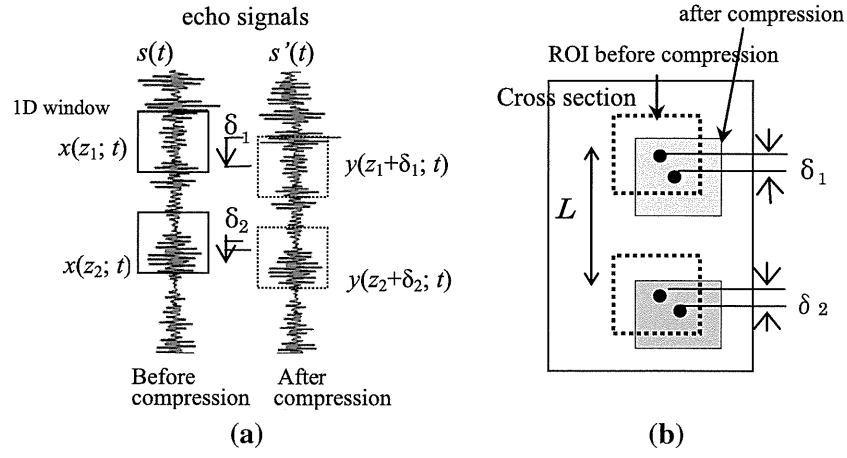


Fig. 7 Calculation of correlation using the spatial correlation method.
a Correlation in 1D window.
b Correlation in 2D window

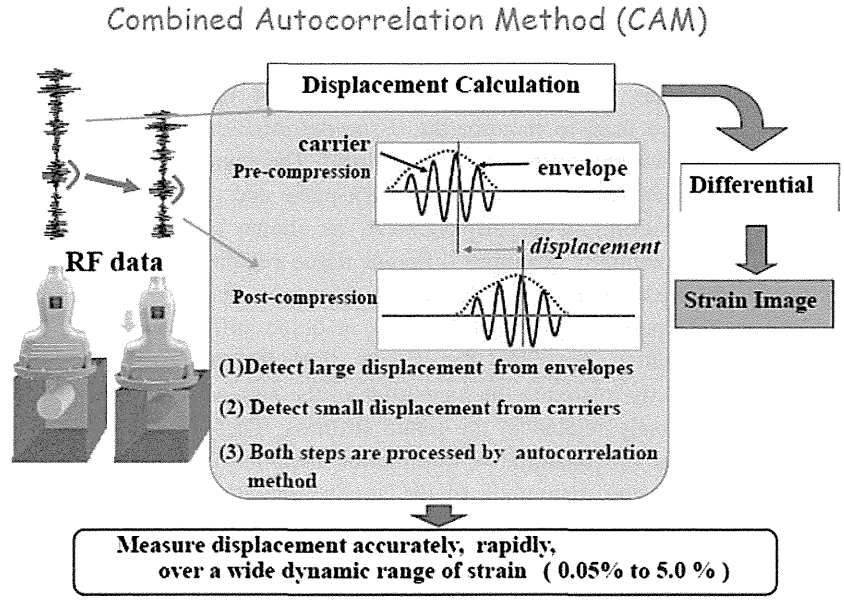
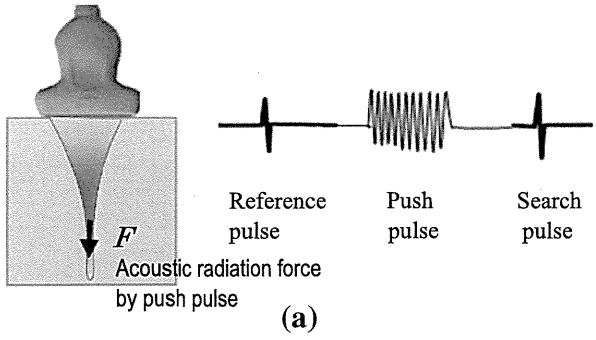


Fig. 8 Principle of the combined autocorrelation method



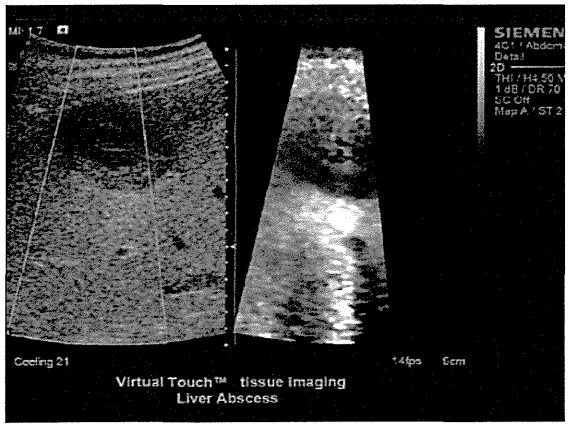


Fig. 9 Pulse sequence in ARFI imaging and clinical example. **a** Acoustical radiation force by push pulse and pulse sequence in ARFI imaging. ARFI imaging utilizes acoustic push pulses and imaging pulses before and after application of the "push" pulse to monitor the tissue deformation (displacement) within the region of the "push." **b** Clinical example (liver abscess)

correcting the rough displacement as shown in Fig. 8. As a result, this method has a wide dynamic range and provides high accuracy, being able to accommodate strain ranging from about 0.05 to 5 % without causing aliasing errors, for not only displacements of less than a wavelength but also large displacements. Since both processes use the autocorrelation method applied in color Doppler, it achieves high speed, high accuracy, and a wide dynamic range for detecting displacement; as a result, the method is suitable for manual compression. Therefore, it was installed in the first equipment that was put to practical use.

In the case of actual equipment, improvements have been made to the above-mentioned spatial correlation method and phase difference detection method, and they are being used in practical equipment, but differences in their characteristics manifest as differences in frame rate, image quality, measurement conditions, etc.

Acoustic radiation force impulse (ARFI) imaging

While strain elastography relies on manual application of pressure, a strain imaging method that deforms tissue using focused acoustic radiation force has also been devised. This method, called acoustic radiation force impulse (ARFI) imaging [22, 23], became commercially available within the past few years.

In this method, a focused acoustic radiation force "push" pulse is generated according to Eq. (15) [24, 25].

$$F = \frac{2\alpha I}{c}, \tag{15}$$

where α is the absorption coefficient, I is the temporal average intensity of ultrasound, and c is the speed of sound.

Imaging pulses before and after application of the "push" pulse are used to monitor the tissue deformation (displacement) within the region of the "push." The same transducer is used both to generate the push pulse and to monitor the resulting tissue displacement, as shown in Fig. 9a. By sequentially scanning different tissue regions with the focused radiation force, images of tissue displacement that portray relative differences in tissue stiffness are generated (Fig. 9b). The tissue displacement response is directly related to the magnitude of the applied force, and inversely related to the tissue stiffness. These images do not provide quantitative information about tissue stiffness because the magnitude of the applied radiation force varies with tissue attenuation from patient to patient and is difficult to quantify. This imaging approach is implemented commercially as the Siemens Virtual Touch^{TM} feature [26–29].

Appropriate measurement conditions and artifacts in strain imaging

Many manufacturers provide elastography equipment offering strain imaging. In terms of artifacts in strain imaging, it should be noted that commonly the stress distribution is not uniform within the body and the tissue elasticity is nonlinear.

As expressed by Eq. (11), strain is used as an index of stiffness instead of the Young's modulus, under the assumption that the stress is uniform. However, in practice, stress tends to concentrate on curved boundaries, making such areas appear softer than adjacent areas, as shown in Fig. 10. In many cases, this kind of artifact is easily recognized based on a priori information such as tumor shape.

Regarding tissue nonlinearity, in the case of biological tissue, the Young's modulus tends to increase when the compression is intensified, as shown in Fig. 11, and the extent of the increase differs from tissue to tissue [30].

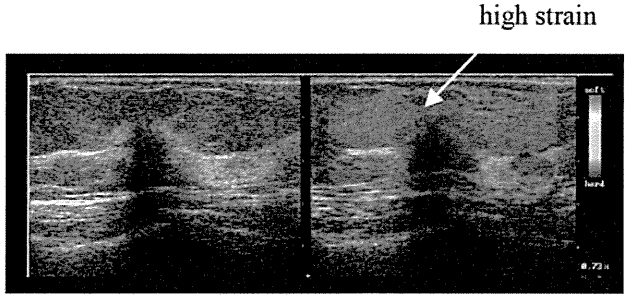
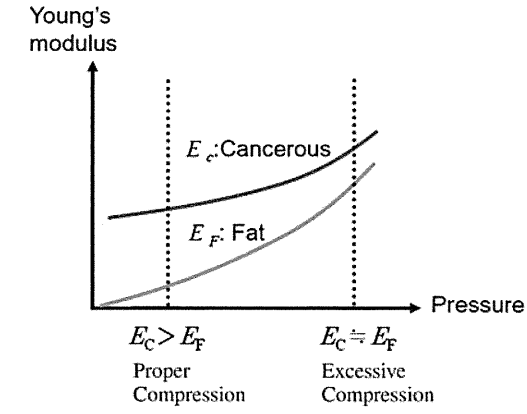
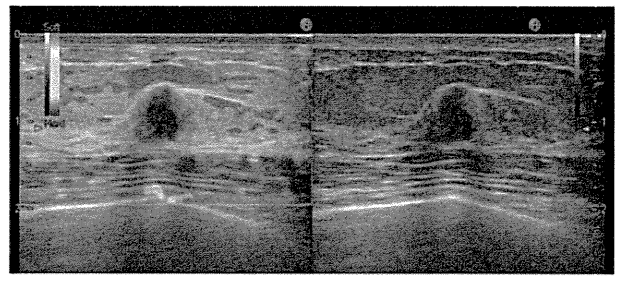


Fig. 10 Artifacts due to stress concentration: breast cancer (scirrhous)



(a)



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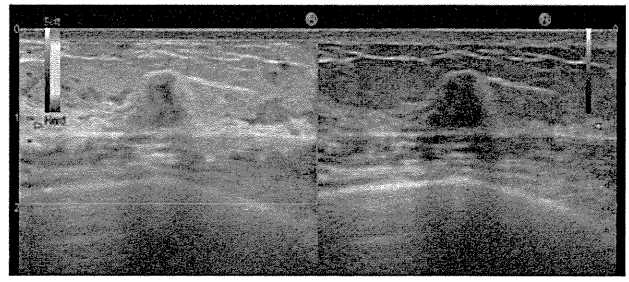

(c)

Fig. 11 Effect of excessive compression (breast cancer). **a** Change in contrast according to nonlinearity of tissue elasticity. When compression is increased, the tissue stiffens and the contrast between fat and a cancerous mass decrease. **b** Adequate compression. **c** Excessive compression

For example, when the degree of compression is slight, the difference in the Young's modulus between mammary gland tissue and tumor tissue is large and consequently the tumor tissue is clearly displayed as a relatively low-strain region, as shown in Fig. 11b. However, when the compression is too strong, the stiffness of the mammary gland will increase, and the difference from the tumor tissue will be smaller, possibly resulting in a false-negative finding, as in Fig. 11c.

Such nonlinearity becomes marked when the compression generates strain in excess of several percent. However, when strain of about 1 % is generated in the mammary gland, stability and reproducibility can be achieved even if

the level of compression fluctuates, since it is within the linear range.

Unlike strain elastography based on manual compression, the ARFI imaging approach does not rely on the transducer compression technique, and it has the advantage of being able to focus the "push" within deep-lying organs, where it can be difficult to generate deformation by compression from the body surface. On the other hand, this method can be depth limited, with most commercial implementations reaching a maximum depth of only about 75 % of corresponding B-mode images from a given transducer.

In addition, in order to generate detectable levels of strain, the "push" pulses are of longer duration than regular diagnostic pulses. Therefore, in order to maintain acoustic output within diagnostic limits, current equipment enforces a certain amount of "cooling" time after each measurement, which reduces the frame rate achievable by this imaging technique. Moreover, it is recommended that it not be used in combination with an environment in which a physiological response to sound could easily occur in the body, such as with use of contrast agents [31, 32].

The method that employs acoustic radiation force requires appropriate focusing of pulse waves to apply pressure in order to generate tissue displacement. As such, it can be affected by the inhomogeneous properties of tissue and incident angle of the pushing pulse. In addition, tissue stiffens when the compression applied by the probe is intensified, because of the nonlinearity of tissue elasticity. Therefore, the method employing acoustic radiation force also requires a technique to maintain the optimal conditions.

Shear wave imaging

Shear wave imaging is based on the theory that the speed of shear wave propagation, c_s, through tissues is related to their stiffness [33]. The relation is expressed by Eq. (12); that is, the elastic modulus, E, is proportional to the square of the speed of shear wave propagation, $E = 3\rho c_s^2$, on the assumption of simple, i.e., linear, isotropic, incompressible, and homogeneous, material.

Shear waves can be generated by a variety of sources, including external vibration, physiological motion, and acoustic radiation force.

Transient elastography

In this method, short pulsed and low-frequency (about 50 Hz) vibrations such as those shown in Fig. 12a are applied at the body surface using vibration excitation [34]. The summed contributions of the transversely polarized shear waves coming from subsources on the body surface give rise to a globally longitudinally polarized shear wave on the axis of the vibrator within the body. The displacements induced in the medium by

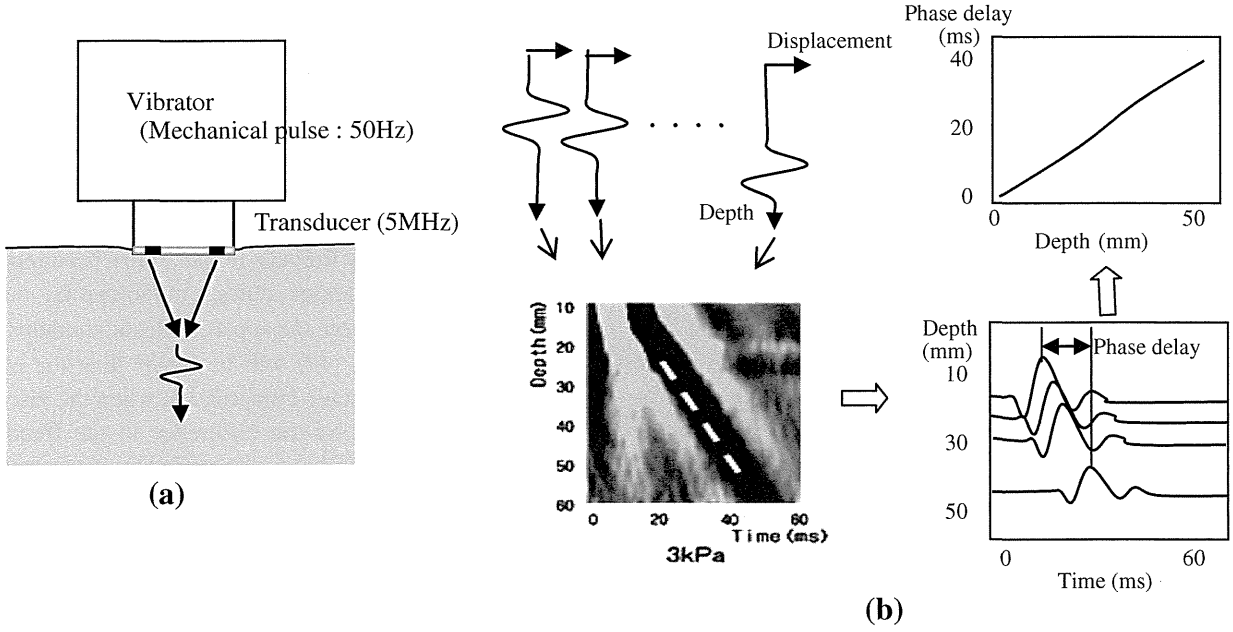

Fig. 12 Principle of transient elastography. **a** Generation of shear waves by mechanical pulse waves. The summed contributions of the transversely polarized shear waves coming from subsources give rise to a globally longitudinally polarized shear wave on the axis of the vibrator. **b** M-mode image of displacement estimates and estimation of shear wave speed

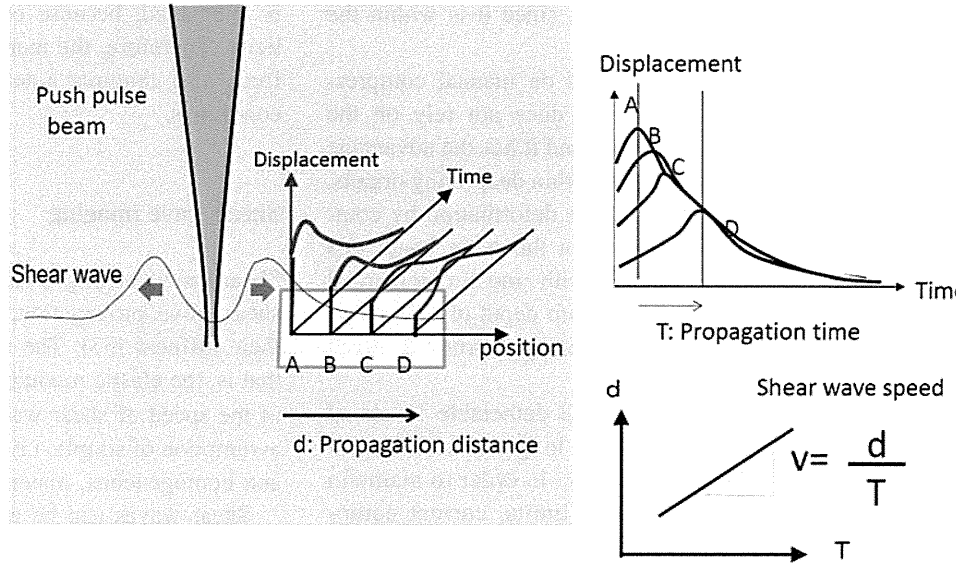

Fig. 13 Measurement of propagation speed of shear waves

this wave are measured by using an approach similar to the Doppler method. M-mode images are composed of many radio frequency (RF) lines stored while the shear wave propagates inside the medium, as shown in Fig. 12b. The phase delay of the shear wave (shear wave propagation time) is obtained from the peak point of this displacement, and the shear wave propagation speed c_s (m/s) is obtained based on the slope of the relation between phase delay and depth. Using ρ (kg/m^3) as the density, the elasticity (Young's modulus) E (kPa) is calculated as $E = 3\rho c_s^2$. This method is currently used in FibroScan^{TM} for evaluation of liver fibrosis.

Shear wave elastography

In response to focused acoustic radiation force excitation, in addition to vibrating at the ultrasonic frequency, the tissue within the region of excitation (ROE) is also deformed, and shear waves are created and propagate away from the ROE in a direction orthogonal to the excitation beam, as shown in Fig. 13.

The right of Fig. 13 shows the displacement through time profiles at the focal depth of radiation force excitation at four different lateral positions. By comparing this waveform between adjacent positions, the propagation time T is obtained. Shear wave speed estimates are generated by calculating the ratio of distance, d, to propagation time, T. In fact, for improved accuracy, the speed is obtained based on the slope of an approximation line on a graph of distance versus propagation time for a measurement point obtained using multiple detection beams within a sample volume.

Since the acoustic radiation force is applied at a single focal location, imaging requires generation of shear waves at multiple points throughout the imaging area, which reduces the frame rate. Another approach is to use a multifocal zone configuration in which each focal zone is interrogated in rapid succession, leading to a cylindrically shaped shear wave extending over a greater depth, enabling real-time shear wave images to be formed as shown in Fig. 14. This approach has been termed supersonic shear imaging, as implemented by ShearWave^{TM} elastography (SWE) [35].

Whichever generation method is used, shear wave imaging can be used to calculate the distribution of c_s. Making use of its quantitative capability, it is used not only for imaging but also for numerically calculating tissue elasticity. Although it is a merit of shear wave imaging that the elastic modulus is obtained without estimating stress, it is to be noted that Eq. (12) relies on the assumption of a simple, i.e., linear, isotropic, incompressible, and homogeneous, material.

At the same time, the apparent elasticity associated with the propagation speed will change with internal pressure caused by blood pressure, etc., which may be different from the elasticity caused by fibrosis. In fact, it has been reported that the velocity increases during inflammation due to jaundice [36]. In addition, the apparent elastic modulus $G = \rho c_s^2$ calculated by Eq. (10) will be larger than the elastic modulus obtained by static compression when the viscosity is high. Figure 15 shows the difference in the frequency dependence of velocity due to the presence of viscosity.

Appropriate measurement conditions and artifacts in shear wave imaging

In shear wave imaging, the Young's modulus is estimated by generating shear waves in the body and measuring their propagation speed. Therefore, the measurement conditions

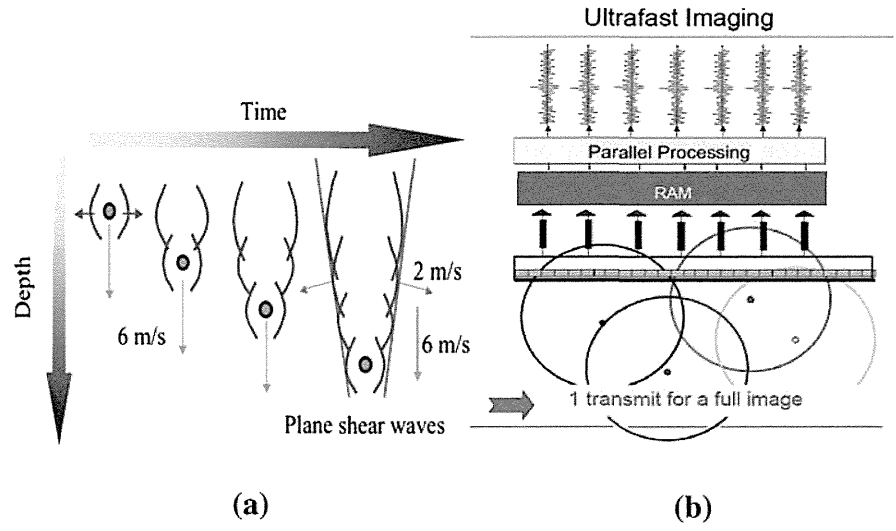

Fig. 14 Shear wave excitation and high-speed measurement by SWE. **a** Formation of the shear wave wavefront by movement of the push pulse focal point, leading to a cylindrically shaped shear wave extending over a greater depth. **b** High-speed measurement of displacement by transmitting plane waves and simultaneously performing aperture synthesis with about 5,000 frames/s, an almost 100-fold increase

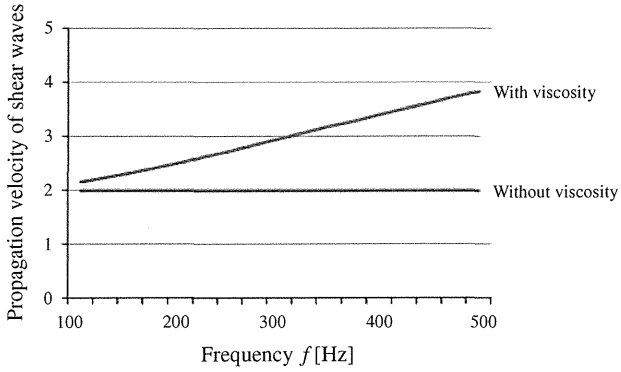

Fig. 15 Difference in propagation velocity according to presence or absence of viscosity ($G = 4$ kPa, $\mu = 3$ Pa s)

to be considered for appropriate imaging and possible artifacts in a clinical setting are as follows:

Insufficient intensity of acoustic radiation force

The method of generating shear waves by tissue compression using an acoustic radiation force requires that pulse waves for excitation appropriately form a focal point in the body. Therefore, when contact between the probe and the surface of the body is inadequate, or when the heterogeneity of acoustic properties of tissue is large, sufficient acoustic force for excitation cannot be achieved at the affected area, resulting in shear waves not being appropriately generated. This causes artifacts and failed measurements.

Effects of refraction/reflection of shear waves

As described in the "Dynamic properties and shear wave speed" section, the range of the distribution of shear waves in tissue is large, i.e., 1–10 m/s, unlike longitudinal waves used in B-mode ultrasound, which travel at approximately the acoustic velocity in water, i.e., about 1,500 m/s. This means that shear waves are strongly refracted at tissue interfaces where the acoustic speed differs. Likewise, the difference in the acoustic impedance of tissue to shear waves is also large, sometimes resulting in the reflection coefficient at a tissue margin being large compared with that for longitudinal waves.

In the case of malignant tumors in particular, the acoustic velocity changes at the tumor site because it is stiffer relative to the surrounding tissue, and the inside of the mass often has a heterogeneous structure and properties; therefore, the wave phenomena caused by refraction and reflection are liable to become marked.

The change in propagation direction caused by refraction must be determined in order to accurately calculate the acoustic velocity of shear waves to obtain the Young's modulus using shear wave imaging. For actual diagnostic equipment, simpler approaches, such as assuming the propagation direction, are applied to emphasize their real-time capability, which is the advantage of ultrasonography. Therefore, although shear wave imaging yields elastic characteristics as numerical values, at the present time, they need to be interpreted carefully, with the understanding that artifacts and variations in measurements will occur.

Relationship between strain and shear wave speed images

Both strain and shear wave speed images provide information related to underlying tissue stiffness. As such, in the absence of artifacts, the correlation between these image types in a given patient is expected to be high. However, when the simplifying assumptions used to derive the

images for the different methods do not accurately reflect the tissue properties, the information provided by each image will be different. One of the important factors is the different frequency range. Strain elastography based on manual compression basically provides static biomechanical properties of tissue, while shear wave imaging represents dynamic properties at a higher frequency range. As a result, there is a possibility that the values of the apparent elastic modulus obtained based on their simple assumptions are different.

Tissue nonlinearity is associated with decreased strain contrast (Fig. 11) and increased shear wave speeds when excessive transducer compression is applied. As a result, for both strain and shear wave imaging, minimizing the amount of transducer compression used during imaging (i.e., to <1 %) will result in the most reproducible imaging scenario. Tissue heterogeneities will also impact both approaches, leading to artifacts arising from reflected waves in shear wave speed images, and strain concentrations surrounding tissue heterogeneities. More detailed investigation of the impact of these assumptions and associated image artifacts should be carried out in the future.

Conclusions

The first practical equipment for elastography was released in 2003, and today every manufacturer offers strain elastography, with many offering both strain and shear wave-based approaches. In addition to breast examination, elastography is now evolving into diagnosis in wider clinical fields including in breast, liver, thyroid, prostate, etc.

On the other hand, it should be noted that there remains an unexplained feature which is required to build the clinical value of elastography and develop more useful equipment, namely disease models that link genetic, cellular, biochemical, and gross pathological changes with observations of biomechanical properties; For example, it is less well understood how biomechanical properties change and are reflected in elasticity images as cancer develops. Development of elastography technology and elucidation of the relation between elasticity images and tissue diseases are connected to each other, since reliable data are provided by high-performance equipment.

At present, as described herein, there are different methods of elastography, each of which has both advantages and weak points. At the same time, this is still an evolving technology and the near future will bring an expanding set of techniques, such as combinations of different methods with strong ability to provide more quantitative and high-resolution images or new diagnostic information related to the biomechanical properties of tissues.

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Hepatology Research

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Review Article

Clinical significance of therapy using branched-chain amino acid granules in patients with liver cirrhosis and hepatocellular carcinoma

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The liver is the major organ for the metabolism of protein, fat and carbohydrate. A nutritional approach is required in the treatment of cirrhosis, which is frequently complicated with protein–energy malnutrition. Several advanced treatment approaches for hepatocellular carcinoma (HCC) have been established in the past decade. HCC is often complicated by cirrhosis, so treatment of the underlying liver diseases is also necessary to improve the prognosis. Branched-chain amino acid (BCAA) granules were developed originally for the treatment of hypoalbuminemia associated with decompensated cirrhosis. However, subsequent studies found various other pharmacological actions of this agent. We review the clinical significance of therapy using BCAA granules in patients receiving different treatment approaches for cirrhosis and HCC based on the published work as well as our own data.

Key words: branched-chain amino acid granules, hepatocellular carcinoma, liver cirrhosis, liver function, recurrence

INTRODUCTION

THE LIVER IS the major organ for the metabolism of protein, fat and carbohydrate.[1,2] Cirrhosis, which develops over a long period of time, is frequently complicated with protein–energy malnutrition (PEM).[1,2] Patients with cirrhosis-associated PEM starve even after a short period of fasting because of the increased energy consumption and decreased glycogen-storage capacity of the liver. The body consumes the endogenous fat as an energy substrate instead of carbohydrate. As a result, fasting hypoglycemia and postprandial hyperglycemia typically occur.[1–4] PEM affects the prognosis by increasing the risk of cirrhosis-associated events and deteriorating the patient's quality of life (QoL), so nutritional treatment is absolutely crucial.[1–3]

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The treatment of hepatocellular carcinoma (HCC) has improved appreciably in the past 20–30 years. The current treatment for HCC with established efficacy is: (i) hepatectomy/liver transplantation; (ii) transcatheter arterial chemoembolization (TACE); (iii) percutaneous radiofrequency ablation (RFA); (iv) percutaneous ethanol injection; (v) percutaneous microwave coagulation therapy; and (vi) molecular-targeted therapy (e.g. sorafenib).[5–9] The most suitable treatment should be selected for individual patients based on thorough evaluation of HCC stage (tumor factor) and hepatic functional reserve.[5–10] In general, HCC develops after cirrhosis associated with viral hepatitis or alcoholic liver disease, so treatment of the underlying liver diseases is no less important than HCC treatment.[5–9,11] Preserving adequate hepatic reserves is necessary after HCC recurrence, which is quite frequent no matter how successful the initial radical treatment for HCC.[12–16]

Branched-chain amino acid (BCAA) granules (Livact; Ajinomoto Pharma, Tokyo, Japan) contain L-valine, L-leucine, and L-isoleucine at a ratio of 1.2:2:1. L-Leucine induces albumin synthesis in hepatic cells via transcription factors such as mammalian target of rapamycin.[1–3,17] BCAA granules were developed originally for the treatment of hypoalbuminemia associated

Table 1 Pharmacological effects of branched-chain amino acid granules

1. Improvement of hypoalbuminemia
2. Improvement of liver cirrhosis-related complications
3. Improvement of insulin resistance
4. Improvement of oxidative stress
5. Improvement of fatty acid metabolism
6. Activation of immune function
7. Suppression of angiogenesis
8. Suppression of liver carcinogenesis

with decompensated cirrhosis. However, subsequent studies found various other pharmacological actions of this drug. Therapy using BCAA granules improves hypoalbuminemia.[16–19] In addition, such therapy also inhibits cirrhosis-related complications such as esophageal varices and ascites,[17,18,20] reduces insulin resistance[17,21,22] and oxidative stress,[17,23] improves fatty-acid metabolism,[17,24] stimulates the immune system,[17,25,26] and inhibits angiogenesis.[17,21,27] The most noteworthy pharmacological action of BCAA granules, however, is the inhibition of hepatic carcinogenesis (Table 1).[17,19,20,22,27–29] Based on the significant inhibition of hepatic carcinogenesis observed after therapy using BCAA granules in patients with liver cirrhosis with a body mass index of 25 kg/m^2 or more shown in a multicenter, randomized, placebo-controlled study (the Lotus Study), the 2010 guidelines for comprehensive treatment of hepatitis virus-related cirrhosis in Japanese patients recommend the use of BCAA granules to preserve liver function and inhibit hepatic carcinogenesis.[16–19,28,30] Conversely, the American Society for Parental and Enteral Nutrition (ASPEN) and the European Society for Clinical Nutrition and Metabolism recommend that BCAA supplementation be carried out only in cirrhotic patients with chronic hepatic encephalopathy that is refractory to pharmacotherapy.[31,32]

Here, we review the clinical significance of therapy using BCAA granules in different treatment approaches for cirrhosis and HCC (i.e. hepatectomy, liver transplantation, RFA, TACE and molecular-targeted agents) mainly based on the published work as well as our own data published between 1997 and 2013. We searched the published work in the PubMed database, and the search strategy was based on the following terms: "branched-chain amino acid", "liver cirrhosis", "liver function", "complication", "clinical outcome", "carcinogenesis", "hepatocellular carcinoma", "recurrence", "hepatectomy", "liver transplantation", "RFA", "TACE" and "molecular-targeted therapy".

Significance of cirrhosis treatment with BCAA granules

In cirrhotic patients, the plasma level of BCAA is positively correlated with the serum albumin level. Such a correlation is seen only in patients with chronic liver diseases such as cirrhosis. The albumin–BCAA correlation and the inability of cirrhotic patients to maintain an adequate plasma level of BCAA with diet alone serve as the theoretical rationale for the use of BCAA granules for the treatment of cirrhosis. In cirrhotic patients, BCAA uptake in skeletal muscle is increased for ammonia detoxification and energy production and, in turn, the plasma level of BCAA and albumin production decrease.[1–3]

Yatsuhashi *et al.* conducted a prospective multicenter study in 204 patients with decompensated cirrhosis and reported a mean increase in the serum albumin level of 0.2 g/dL after 6 months of treatment with BCAA granules as well as a significant increase in the serum albumin level in patients with intake of a poor diet (poor intake of energy).[33] Therapy using BCAA granules also significantly decreased the incidence of ascites even in patients with an unchanged serum albumin level because of qualitative improvement of the serum albumin level (specifically, an increase in the level of reduced albumin and decrease in the level of oxidized albumin).[33–35]

The importance of treatment compliance was suggested in a study conducted by Takaguchi *et al.*[36] That prospective, large-scale, multicenter, observational study in 2894 patients with decompensated cirrhosis reported that the incidence of cirrhosis-associated events was decreased significantly in patients with good adherence to BCAA treatment compared with those with poor adherence. The authors emphasized the importance of thorough instruction regarding medications to patients.[36]

The appropriate timing of the initiation of BCAA treatment is controversial. The approved indication of BCAA granules in Japan is for the treatment of decompensated cirrhosis in patients with a serum albumin level of 3.5 g/dL or less, and the Japanese Nutritional Study Group for Liver Cirrhosis has also recommended that BCAA granules should be administrated in cirrhotic patients with a serum albumin level of 3.5 g/dL or less, Fisher's ratio of 1.8 or less and/or BCAA : tyrosine ratio (BTR) of 3.5 or less.[37] Hence, therapy using BCAA granules is, in general, started when the serum

albumin level is 3.5 g/dL or less in clinical settings.[11,37] However, earlier initiation of BCAA treatment has been attempted in cirrhotic patients with a serum albumin level of 3.6 g/dL or more. Habu *et al.* classified their patients into four treatment arms based on their serum albumin level and the BTR.[38] The decrease in the serum albumin level was inhibited after therapy using BCAA granules even in patients with a serum albumin level of 3.6 g/dL or more if their BTR was 4 or less, so the authors highlighted the usefulness of early intervention with BCAA granules.[38,39] A prospective, multicenter study in Japanese patients with hepatitis C virus-related decompensated cirrhosis with a serum albumin level of 3.6 g/dL or more complicated with insulin resistance (BCAA Granules for patients with Hepatitis C virus-related Liver Cirrhosis and Insulin Resistance on the Effect of Reduction of Carcinogenic Risk in the Liver [BLOCK] study, Japan Liver Oncology Group [JLOG] 1004 Trial) is ongoing. If the superiority of therapy using BCAA granules is demonstrated in that study, BCAA granules will become available for a wider range of cirrhotic patients.

As mentioned above, BCAA granules can inhibit hepatic carcinogenesis.[17,19,20,22,27–29] Several reports have focused on the usefulness of BCAA granules for the inhibition of liver carcinogenesis through improvement of insulin resistance.[17,21,22] Insulin and insulin-like growth factor (IGF) can promote the growth of HCC.[40] Kawaguchi *et al.* reported that BCAA granules suppress liver carcinogenesis through amelioration of insulin resistance via: (i) BCAA activation of the insulin signaling cascade through upregulation of phosphatidylinositol 3-kinase with reduction of serum insulin levels; and (ii) inhibition of the IGF/IGF-1 receptor axis by suppressing the expressions of IGF-1, IGF-2 and IGF-1 receptor mRNA.[17,41] They also reported that the improvement of insulin resistance by BCAA granules may be related to the migration of HCC, suppression of angiogenesis and epithelial–mesenchymal transition of hepatocytes, and that BCAA granules may inhibit liver carcinogenesis (at least in part) by reduction of oxidative stress and strengthening of immune functions.[17]

There are several reports of the usefulness of BCAA supplementation on the QoL of patients with liver cirrhosis.[42,43] Kawamura *et al.* demonstrated that, in 453 patients with chronic liver disease, QoL decreased significantly according to the progression of disease as assessed by the scores from Short Form 36 ($P < 0.05$) and that the QoL of patients with chronic liver diseases was improved in the BCAA granules administration group ($n = 13$) compared with the control group ($n = 12$) after 6 months.[42] Hepatic encephalopathy (HE) is a major complication in patients with liver cirrhosis that is related to a poor prognosis and poor QoL.[44] Sleep disturbance may be associated with minimal HE.[45] Les *et al.* conducted a randomized study involving 116 patients who had experienced an episode of HE (58 patients in the BCAA group and 58 patients in the maltodextrin group) to examine the effect of BCAA: they reported that supplementation with BCAA improves minimal HE and muscle mass.[43] Tryptophan, which is a precursor of the neurotransmitter 5-hydroxytryptamine (which is related to sleep disturbance), may be regulated by BCAA supplementation.[46]

With the wide range of pharmacological actions, such as increasing the serum albumin level,[16–19] inhibiting cirrhosis complications/angiogenesis/hepatic carcinogenesis,[17–20,22,27–29] improving insulin resistance[17,21,22] and fatty-acid metabolism,[17,24] reducing oxidative stress,[17,23] and increasing stimulation of the immune system,[17,25,26] therapy using BCAA granules may be an indispensable treatment for cirrhosis.

Significance of BCAA granules in different approaches to HCC treatment

Hepatectomy

Along with liver transplantation, hepatectomy is a curative treatment approach for HCC.[6,8,9,47–49] According to guidelines set by the European Association for the Study of the Liver (EASL), hepatectomy is indicated in patients with a single tumor of 2 cm or less in diameter, performance status (PS) 0, Child–Pugh class A and no portal hypertension.[50] In Japan, however, hepatectomy is considered in patients with three or less tumors of less than 3 cm in diameter, no vascular invasion, Child–Pugh class A or B, and expected tolerance to surgery, or even in those with four or more tumors of more than 3 cm in diameter and vascular invasion if they are expected to tolerate surgery and the treatment may improve the prognosis.[51] Hepatectomy is considered the first-line initial treatment for resectable HCC because of generally good surgical outcomes and poor availability of brain-dead liver donors in Japan.[52,53]

In HCC patients in whom a large volume of liver has been removed and in those with concurrent cirrhosis, the hepatic functional reserve is expected to decrease after resection. In several studies, the serum albumin level has been identified as a contributing factor for the prolonged postoperative survival time in HCC patients.[13,54–57] Thus, nutritional treatment with BCAA granules would be an essential approach based on this

observation as well as the fact that BCAA therapy prevents perioperative complications.

Togo *et al.* reported, in their study in 43 HCC patients with advanced cirrhosis, that post-hepatectomy treatment with BCAA granules inhibited the progression of cirrhosis and improved the prognosis.[58] The usefulness of oral nutritional supplements to prevent post-hepatectomy hepatic failure[59] and the usefulness of BCAA granules to inhibit postoperative HCC recurrence[29] have also been reported. Ichikawa *et al.* reported, in their prospective study in 56 HCC patients aged 65 years or more, that post-hepatectomy HCC recurrence was suppressed significantly and that the postoperative clinical course was more favorable in the BCAA treatment group ($n = 26$) compared with the regular-diet group ($n = 30$).[29]

Treatment with BCAA granules has appreciable clinical significance in HCC patients (especially those with underlying advanced cirrhosis) in terms of preserving hepatic functional reserve, preventing perioperative complications and inhibiting postoperative recurrence.

Liver transplantation

As an important choice of HCC treatment in western countries,[8,60,61] liver transplantation is considered even in patients with decompensated cirrhosis of various causes.[62] Assuming that the Milan criteria are satisfied, living donor partial liver transplantation for the treatment of decompensated cirrhosis complicated by HCC has been covered by the national health insurance system in Japan since 2004.[63] As described above, living donor liver transplantation is the major choice of treatment because of the shortage of brain-dead donors in Japan.[8,60,61,63,64]

The usefulness of BCAA granules in patients who have undergone liver transplantation has been reported in two studies.[65,66] In a prospective randomized study in 56 Child–Pugh class A cirrhotic patients without major complications, Kawamura *et al.* reported that early intervention with BCAA granules significantly decreased cirrhosis-related complications and prolonged the time to liver transplantation.[65] In a retrospective study in 236 patients who underwent living donor liver transplantation, Shirabe *et al.* reported a significant decrease in post-transplantation septic complications in patients pretreated with BCAA granules.[66] Considering the global shortage of liver donors,[6–9] BCAA granules could be a promising treatment for subjects undergoing liver transplantation.

Percutaneous treatment

Since its introduction in Japan in 1999, RFA has rapidly gained popularity because of its excellent antitumor effect and low extent of invasiveness. Percutaneous RFA is the first-line percutaneous treatment for HCC.[5–9,11,14,67–72] EASL guidelines recommend percutaneous RFA for HCC of PS 0–2, Child–Pugh class A or B, and three or less unresectable tumors of 3 cm or less in diameter. In Japan, percutaneous RFA is, in general, indicated for patients of Child–Pugh class A or B and three or less unresectable tumors of 3 cm or less in diameter. Even in patients with unresectable tumors of 3 cm or more in diameter, percutaneous RFA in combination with TACE is recommended to expand the ablated area.[50,51,73]

Percutaneous RFA is less invasive than hepatectomy, but hepatic functional reserve may decrease after RFA in some patients.[74–76] The possible causes of a postoperative decrease in the serum albumin level include: (i) decreased albumin synthesis secondary to hepatocyte decrease; (ii) inhibition of albumin synthesis by inflammatory cytokines; and (iii) loss of protein due to inflammation at the ablation site.[74–76] We reported the association between the serum albumin level and survival of HCC patients treated with percutaneous RFA, so therapy using BCAA granules may be a useful treatment for RFA-treated HCC frequently complicated by cirrhosis.[11,67]

One of the disadvantages of percutaneous RFA is the high prevalence of recurrence of HCC.[6,8,9,15,48,67] We found the prevalence of HCC 5 years after RFA to be approximately 80% even in patients with a single HCC.[67] The regimen to prevent HCC after RFA includes antiviral therapy (interferon therapy for hepatitis C and nucleoside analog therapy for hepatitis B) and liver-support therapy to keep the hepatic enzymes at a low level.[67,77–83] BCAA granules with potential anticarcinogenic effects may also be useful for preventing HCC recurrence post-RFA.[11,27]

Yoshiji *et al.* focused on the inhibitory action of BCAA granules and an angiotensin-converting enzyme inhibitor (ACE-I) against angiogenesis, and evaluated the effect of these agents in preventing post-RFA recurrence of HCC in a prospective randomized study.[27] The post-RFA prevalence of HCC and levels of vascular endothelial growth factor were decreased significantly in the combined BCAA granules and ACE-I treatment group compared with the control group, suggesting a possible synergistic effect of the two drugs to inhibit HCC recurrence after RFA.[27] Our retrospective controlled study in 256 HCC patients with a serum albumin level of

3.5 g/dL or less treated with percutaneous RFA showed significantly higher overall and recurrence-free survival in patients treated with BCAA granules ($n = 115$) compared with those receiving a regular diet ($n = 141$).[11] The use of BCAA granules was identified as a contributing factor to prolonged survival in a multivariate analysis.[11] The mechanism of the inhibitory effect of BCAA granules against HCC recurrence after RFA needs to be verified in a large-scale prospective study. BCAA granules may inhibit HCC recurrence in patients who have undergone percutaneous RFA as well as in those who have undergone hepatectomy.[11,29]

TACE

Transcatheter arterial chemoembolization is a combination of local chemotherapy through feeding blood vessels and the use of embolizing material.[16,84–87] TACE is most frequently used for the treatment of HCC in Japan, where it was originally developed.[84,87–90] EASL guidelines recommend TACE for unresectable, Child–Pugh class A or B multiple HCC with no vascular invasion, whereas in Japan the therapy is recommended even for HCC with vascular invasion if it is Vp1 or Vp2.[50,51]

The factors affecting the survival of HCC patients treated with TACE are: (i) tumor stage; (ii) tumor markers; and (iii) hepatic functional reserve.[84] Preserving hepatic functional reserve is a critical issue in HCC patients who, in general, are treated repeatedly with TACE.[16,88–92] However, in some patients, hepatic functional reserve decreases after TACE because of complications such as post-TACE syndrome.[93]

The usefulness of BCAA granules or BCAA-enriched "snacks" for patients with unresectable HCC treated with TACE has been suggested in several studies.[16,91,92] In a randomized controlled trial (RCT) in 56 HCC patients treated with TACE, Takeshita *et al.* found that the post-TACE decrease in liver function was suppressed significantly in patients who received an enteral nutritional formula for hepatic failure given as a late-evening snack (LES) compared with the control group.[91,94] Our retrospective controlled study in 99 HCC patients treated with TACE showed that therapy using BCAA granules significantly inhibited the decrease in hepatic functional reserve at 3 months and 6 months compared with the regular diet group.[16] According to EASL guidelines, if HCC with Child–Pugh class B treated with TACE recurs as Child–Pugh class C, TACE is not indicated for the recurred HCC. The significance of therapy using BCAA granules is considerable in terms of permitting repeated TACE.

Molecular-targeted drugs (sorafenib)

There had long been a lack of evidence to support systemic chemotherapy for unresectable advanced HCC.[95] However, after the efficacy of a molecular-targeted drug, sorafenib, for unresectable advanced HCC was demonstrated in two RCT (SHARP trial and Asia–Pacific trial), the drug was approved for the treatment of unresectable advanced HCC in Japan in 2009.[96,97]

The action of sorafenib against tumor growth and angiogenesis is based on the inhibition of the activities of intracellular kinase and receptor tyrosine kinase.[96–106] The new era of systemic chemotherapy for unresectable advanced HCC was started with the introduction of sorafenib.[96–103,106] EASL guidelines recommend sorafenib for unresectable, advanced, Child–Pugh class A or B HCC with PS 0–2 and vascular invasion or distant metastasis.[50] According to Japanese guidelines, sorafenib is recommended for unresectable, advanced, Child–Pugh class A HCC with vascular invasion or distant metastasis as well as for patients intolerant to TACE or in whom the procedure is anatomically unsuitable.[51,104,105]

Several cases of adverse events associated with the use of sorafenib have been reported.[96–106] Patients should be monitored carefully for hepatic dysfunction during sorafenib therapy because decreased hepatic reserve caused by sorafenib may result in irreversible hepatic failure.[102] Even if hepatic failure is avoided, sorafenib treatment may have to be discontinued or the dose reduced.[102] Many HCC patients treated with sorafenib have concurrent cirrhosis.[96–106] Hence, intervention with BCAA granules has appreciable importance in terms of preserving hepatic functional reserve and ensuring continued sorafenib treatment.[107] Our previous study revealed that therapy using BCAA granules significantly inhibited the decrease in serum albumin level and prolonged the duration of sorafenib treatment and survival in patients with a serum albumin level of 3.5 g/dL or less compared with the regular diet group.[107] The synergistic effect of sorafenib and therapy using BCAA granules to inhibit angiogenesis may have contributed to the better prognosis.

There remains a lack of evidence to support the effect of nutritional intervention in patients with unresectable advanced HCC treated with sorafenib. However, therapy using BCAA granules should be considered as a treatment option.

CONCLUSION

WE DISCUSSED THE significance of the use of BCAA granules in the treatment of cirrhosis and

Table 2 Summary of current knowledge of branched-chain amino acid granules for hepatocellular carcinoma (HCC) therapy

1. Prolongation of survival due to the improvement of hypoalbuminemia after HCC therapy
2. Improvement of liver cirrhosis-related complications after HCC therapy
3. Suppression of septic complications due to the activation of immune function after HCC therapy
4. Possibility of suppression of HCC recurrence after HCC therapy

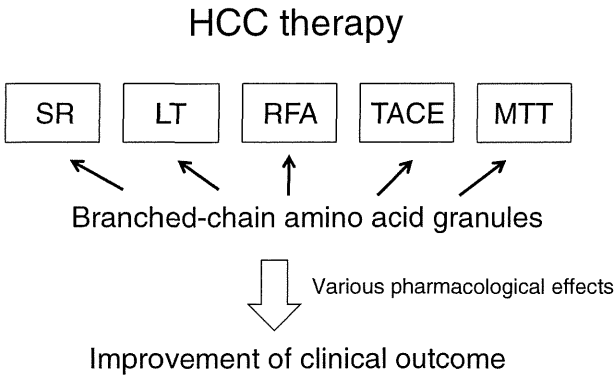

Figure 1 Schematic presentation of the effect of branched-chain amino acid granules for HCC therapy. HCC, hepatocellular carcinoma; LT, liver transplantation; MTT, molecular-targeted therapy; RFA, radiofrequency ablation; SR, surgical resection; TACE, transcatheter arterial chemoembolization.

HCC based on a review of the published work as well as our own data. With a variety of pharmacological actions, BCAA granules are a promising treatment for HCC. (Fig. 1) Summary of current knowledge of BCAA granules for HCC therapy is shown in Table 2.

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Usefulness of Arrival Time Parametric Imaging in Evaluating the Degree of Liver Disease Progression in Chronic Hepatitis C Infection

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Objective—To determine whether the degree of liver disease progression in chronic hepatitis C infection can be evaluated by arrival time parametric imaging using contrast-enhanced sonography with Sonazoid (perfluorobutane; GE Healthcare, Oslo, Norway).

Methods—In this study, 60 patients with liver disease in chronic hepatitis C infection were examined and compared with 10 healthy volunteers who served as controls. A recommended dose of the sonographic contrast agent Sonazoid was intravenously infused, and the S5 or S6 region of the liver and right kidney were observed concurrently while movies of the procedure were saved. Arrival time parametric images of liver parenchymal blood flow were created, with red pixels to indicate an arrival time of 0 to 5 seconds and yellow pixels to indicate an arrival time of 5 to 10 seconds. From the obtained images, the ratio of the red area to the entire enhanced area of the liver was calculated using image-processing software. Each participant was subsequently subjected to liver biopsy for liver fibrosis staging according to Metavir scores, and the determined fibrosis stage was compared with the ratio of red. The serum albumin level, platelet count, and prothrombin time were also compared with the ratio of red for each participant.

Results—The ratio of red increased significantly as liver fibrosis stage advanced ($P < .01$ for F1 versus F2; $P < .01$ for F1 versus F3; $P < .01$ for F1 versus F4; and $P < .01$ for F2 versus F4). As the ratio of red increased, significant decreases were observed in the serum albumin level ($r = -0.29$; $P = .027$), platelet count ($r = -0.46$; $P = .0003$), and prothrombin time ($r = -0.46$; $P = .0002$).

Conclusions—Arrival time parametric imaging using Sonazoid-enhanced sonography enables noninvasive evaluation of the degree of progression of liver disease in chronic hepatitis C infection and is thus considered clinically useful.

Key Words—arrival time parametric imaging; chronic liver disease; contrast-enhanced sonography; hepatitis C virus; liver fibrosis; Sonazoid

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Abbreviations
ROC, receiver operating characteristic

The liver is unique in that it receives its blood supply from two sources: the portal vein and the hepatic artery. In a healthy person, the liver receives approximately 70% to 80% of its blood supply from the portal vein and 20% to 30% from the hepatic artery.[1] Once hepatitis C virus infection occurs, a repeated cycle of necrosis, sloughing, and fibrosis of liver tissue occurs, leading to chronic hepatitis and cirrhosis. As a result of these histologic changes and various other factors, such as production of vasoactive substances and cytokines, the hepatic blood flow balance changes

from "portal vein dominant" to "hepatic artery dominant."[1–4] It would be clinically useful if a quantitative evaluation of the hepatic blood flow balance change could be made by an imaging modality, as doing so would enable noninvasive evaluation of the degree of liver disease progression in chronic hepatitis C infection.

We considered that the best way to understand the liver-specific hemodynamic change is to compare it with that of the kidney, which is supplied exclusively by arteries, and attempted to evaluate the degree of disease progression by analyzing liver-kidney blood flow contrast using contrast-enhanced sonography with Levovist (SH U 508A; Schering AG, Berlin, Germany). However, a detailed evaluation could not be made with Levovist, which provides contrast-enhanced images via destruction of bubbles using a high mechanical index.[5] Sonazoid (perfluorobutane; GE Healthcare, Oslo, Norway), a contrast-enhanced sonographic agent introduced to the market in 2007, enables continuous observation of enhancement patterns without the need for bubble destruction. We analyzed liver-kidney blood flow contrast based on a time-intensity curve obtained by Sonazoid-enhanced sonography, which enabled a more detailed evaluation of the degree of liver disease progression in chronic hepatitis C infection compared with Levovist-enhanced sonography.

However, the time-intensity curve is a graph and is thus not intuitive to or easily interpreted by everyone when used to assess blood flow. It is also problematic that time-intensity curves can readily vary under different testing conditions, such as with a different volume and infusion rate of contrast medium. We thus shifted our focus to arrival time parametric imaging, which visualizes the time taken for the contrast agent to arrive in the target tissue as a superposition of a time-dependent color-mapped image on a B-mode image. With arrival time parametric imaging, the arrival time is not greatly affected by contrast medium infusion conditions. Color mapping also enables us to bidimensionally and intuitively understand tissue enhancement patterns.

The objective of this study was to determine whether we can evaluate the degree of progression of chronic liver disease by arrival time parametric imaging by comparing arrival time parametric imaging findings with the liver fibrosis stage, serum albumin level, platelet count, and prothrombin time for liver disease in chronic hepatitis C infection.

Materials and Methods

Participants

This study examined 60 patients with liver disease in chronic hepatitis C infection who underwent Sonazoid-enhanced sonography at the Division of Gastroenterology and Hepatology of Toho University Omori Medical Center between June 2007 and June 2010 and who had available histopathologic findings from liver biopsy. In addition, 10 healthy volunteers with no biochemical evidence of liver disorders or abnormal findings, such as fatty liver, on B-mode sonography were included as controls. The patients with liver disease comprised 34 men and 26 women, with ages ranging from 22 to 87 years (mean ± SD, 53 ± 12 years). The volunteers consisted of 6 men and 4 women, with ages ranging from 26 to 79 years (mean, 63 ± 15 years). Patients were required to have positive findings for hepatitis C virus RNA, as determined by a quantitative TaqMan (Invitrogen, Carlsbad, CA) polymerase chain reaction, and negative findings for hepatitis B surface antigen and hepatitis B core antibody. Alcoholics with a daily alcohol consumption of 80 g or more and patients with heart or kidney disease, large portal collateral circulations, or liver tumor or portal vein embolisms were excluded from the study. Patients in whom the liver could not be visualized due to narrow intercostal spaces or other reasons were also excluded. This study was performed with approval of the Ethics Committee at Toho University Omori Medical Center (approval number 21-26) and participants' consent to participate in the study.

Contrast-Enhanced Sonography

The sonography system used was an SSA-790A unit (AplioXG; Toshiba Medical Systems, Otawara, Japan) with a 3.75-MHz convex probe (PVT-375BT). Imaging was performed with a low mechanical index of 0.22 to 0.29 and a frame rate of 15 to 18 frames per second. Images showing liver parenchyma from the right intercostal space to the S5 or S6 region of the right hepatic lobe and the right kidney on a single screen were used for analysis. Focus was set at a depth of 6 to 8 cm to visualize the kidney. The participants were examined in the supine position with the right arm elevated above the head and instructed to hold their breath.

Sonazoid was then infused at 0.015 mL/kg (recommended dose) as a bolus via the median cubital vein. Images taken for about 40 seconds immediately after Sonazoid infusion were saved as raw data on the system's hard disk drive. Sonography was performed by a single sonographer to ensure a consistent imaging condition.

Arrival Time Parametric Imaging

Arrival time parametric imaging calculation and drawing were performed with saved movie data by using software