[Article Type]

Original Article (Clinical Original)

[Short title]

Diagnosis of cirrhosis by contrast-enhanced US

[Title]

Noninvasive diagnosis of compensated cirrhosis by analysis of time intensity curve portal vein slope gradient using contrast-enhanced ultrasonography

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[Abstract]

Purpose: We measured vascular time intensity curves (TIC) as slope gradients (SG) of intrahepatic vessels via contrast-enhanced ultrasonography (CEUS). The aims were to assess the diagnostic accuracy of the SG of each hepatic vessel, particularly of the portal vein (PV), for cirrhosis, and to compare this method with conventional modalities.

Methods: Fifty-one preoperative patients underwent CEUS, and TIC was plotted. The SG of the hepatic artery (HA), PV and hepatic vein (HV) were obtained from the linear functions between the slope of the arrival time of the contrast agent and the peak enhancement time of each vessel. Transit times and biochemical markers were also measured. Patients were divided into three groups according to Metavir score: F0/1 group (n = 14), F2/3 group (n = 21) and F4 group (n = 16).

Results: The PVSG was decreased significantly in the F4 group (F0/1: 29.1 ± 2.27, F2/3: 23.1 ± 1.86, F4: 14.7 ± 2.13). The PVSG showed high accuracy in diagnosing cirrhosis and was correlated with ICG-R15 and hyaluronic acid (Spearman rank correlation; ρ = -0.5691, $p < 0.001$ and ρ = -0.4652, $p = 0.0006$).

Conclusions: The PVSG has the potential to be a diagnostic tool to identify

well-compensated cirrhosis.

[Introduction]

Liver cirrhosis is a chronic, diffuse, and progressive condition characterized by fibrosis and conversion of normal liver architecture into structurally abnormal nodules. Although more than 1% of some populations may have histological evidence of cirrhosis, cases of compensated cirrhosis often go clinically undetected for prolonged periods of time [1]. In patients with chronic liver disease, the presence of cirrhosis and degree of fibrosis are important factors as they help in deciding therapeutic options and can direct patient management, particularly in cases in which hepatic resection is indicated for concomitant primary malignancy. Several non-invasive evaluations of chronic liver disease are reported to be useful [2-5] but well-compensated cirrhotics may have normal or near-normal levels of those parameters; thus, those parameters are not effective for evaluation of the degree of liver disease, which is critical for predicting perioperative risk. Although liver biopsy is considered the gold standard for assessing the severity of fibrosis and the presence of cirrhosis, the fact that only one part of the liver is sampled leads to false negative results in up to 30% of cases [6, 7]. Furthermore, biopsy is not without inherent risks and cannot be performed repeatedly in follow-up. Therefore,

there is a need for a simple, reliable, and non-invasive technique to assess hepatic fibrosis and cirrhosis.

Studies have shown that contrast-enhanced ultrasonography (US) has high accuracy in the diagnosis of cirrhosis [8-10]. The time of onset of US contrast enhancement of the hepatic veins (hepatic vein arrival time: HVAT) is reported to be especially useful. A shortening HVAT correlates with increasing severity of liver disease due to arterio-venous shunting and arterializations of capillary beds in the liver. A recent study demonstrated that measuring HV-HA interval time and HV-PV interval time, which correspond to the interval from the arrival time of the contrast agent into the hepatic artery (HA) or portal vein (PV) to the hepatic vein (HV), could differentiate mild fibrosis from more severe degrees of fibrosis in patients with chronic liver disease [11, 12]. However, the HVAT is influenced by intrahepatic circulatory changes rather than extrahepatic hemodynamic changes, which are also important in assessing the severity of liver disease [13].

Liver cirrhosis is characterized not only by changes of intrahepatic circulation but also by extrahepatic hemodynamic changes such as porto-caval and gastrointestinal

shunting, splenic circulatory changes and hypersplenism. These affect the inflow hemodynamics of the PV as well as the HA as a result of the "hepatic arterial buffer response" [14]. On this basis, we measured the intrahepatic vascular intensity curve as a slope gradient (SG) with a contrast agent, Sonazoid (GE Healthcare, Oslo, Norway), focusing on the PV [15, 16].

The aim of this prospective study was to assess the diagnostic accuracy of the SG of each hepatic vessel, particularly of the PV, in detecting and characterizing compensated cirrhosis severity compared with the conventional biochemical modalities. We also assessed the advantage of the SG compared with recently reported transit time analyses with a contrast agent for HVAT, HV-HA interval time and HV-PV interval time in diagnosing compensated cirrhosis with liver tumors.

[Materials and Methods]

Patients

Fifty-one preoperative patients who were referred to our Department of Surgery between May 2009 and February 2010 were enrolled in this study. All patients had liver tumor(s) and were scheduled to undergo hepatic resection or ablation therapy. Patients were excluded if they had (a) previous hepatobiliary-pancreatic surgery, splenectomy, porto-caval shunt operation or TIPS, (b) liver tumor(s) more than 5 cm in size or adjacent to the major portal or hepatic vein (this would affect the hepatic circulation), and (c) chronic renal disease, cardiac dysfunction or chronic obstructive pulmonary disease, all of which induce systemic hemodynamic abnormalities.

Characteristics of the patients were as follows; there were 35 males and 16 females with a mean age of 67.03 years (range, 43-88 years). Twenty-eight were HCV antibody positive. 3 were HBV surface antigen positive. 1 patient had HBV and HCV co-infection. 4 had alcoholic hepatitis, and 2 had cryptogenic hepatitis. All patients were classified as Child-Pugh grade A. The mean size of the patients' tumors was 24.16 ± 8.70 mm, and the mean number of tumors per patient was 1.27 ± 0.45. Informed consent to participate

in this study was obtained for all patients.

Ultrasound examination

All patients were tested in the morning after an overnight fast. One surgeon with over 6 years of experience in US including Doppler US and over 3 years of experience with contrast-enhanced US, who was blind to the clinical data, performed all tests. The ultrasound scanner used was a Toshiba Aplio XG (Toshiba, Tokyo, Japan) with a curved 3.75 MHz transducer. The apparatus setting for the low mechanical index (MI) harmonic imaging was standardized as follows: gain of 80, dynamic range of 50 dB, MI of 0.21, with the focus point 8cm from the surface. In each case, the right hepatic artery (HA), right portal vein (PV), and right hepatic vein (HV) were simultaneously scanned via the right intercostal view. The microbubble contrast agent used was Sonazoid (GE Healthcare, Oslo, Norway). A 23G cannula was inserted into the left antecubital fossa vein of the patient. Sonazoid was injected manually at a dose of 0.0075ml/kg, followed by a rapid normal saline flush (10ml). After injection of Sonazoid, patients were asked to hold their breath for as long as possible (at least 30seconds) and gray scale cine

images were digitally recorded onto the hard disk drive of the US scanner.

Data analysis

Brightness value and time analysis were performed using an off-line personal computer with Clip Washer (Toshiba, Tokyo, Japan) and ImageJ (NIH), which is available free of charge for multiple operating systems at http://rsb.info.nih.gov/ij/. First, we decompressed the cine image saved as an Audio Video Interleave (AVI) format into an uncompressed AVI. In the uncompressed AVI file, the interval of each frame is 1/15 of a second. The 15 frames per second gray scale images were processed with the ImageJ software. We observed the cine image frame-by-frame, and the arrival time of each vessel was set at the time of the first echogenic microbubble observed in the vessel.

We set circular ROIs in the HA, PV and HV and measured the brightness value automatically using ImageJ (Fig. 1). The brightness value in each pixel was expressed as 0 at minimum and 255 at maximum. A brightness level in the ROI of 255 signifies that all pixels in the ROI are completely filled with pixels with a 255 brightness value, which means the established circular ROI is visually filled with contrast agent. After

measuring brightness values in each vessel, we made time intensity curves of the three vessels using Excel (Microsoft, WA, USA) (Fig. 2). Peak enhancement time was evaluated by the time intensity curve (TIC). Then we calculated the gradient of the slope between the arrival time and the peak enhancement time as linear functions by the linear approximation method using Excel. We named the gradient of the obtained linear functions the slope gradient (SG) (Fig. 3).

Histological assessment of specimens

In 33 patients, histological assessment of fibrosis was performed on the resected specimen at the time of surgery for the liver tumor. In 18 patients, histological assessment was performed by intraoperative biopsy using a 17-gauge needle at the time of surgical ablation. In all patients, histological findings were interpreted by two independent pathologists who were blinded to the findings of contrast enhanced US and other clinical data. The stage of fibrosis was evaluated semiquantitatively with the Metavir scoring system [17], as follows: F0 = no fibrosis, F1 = portal fibrosis without septa, F2 = portal fibrosis and few septa, F3 = numerous septa without cirrhosis, and F4

= cirrhosis. The fibrosis staging of all patients was as follows; F0 in 5 (9.8%), F1 in 9 (17.6%), F2 in 13 (25.4%), F3 in 8 (15.6%), and F4 in 16 (31.3%). According to the grade of fibrosis, patients were divided into three groups; F0 or F1 as normal/mild fibrosis (F0/1 group; n = 14), F2 or F3 as moderate/severe fibrosis (F2/3 group; n = 21) and F4 as cirrhosis (F4 group; n = 16). In the F4 group, all patients were classified as Child-Pugh grade A (Table 1).

Statistical analysis

All statistical analyses were conducted with JMP software Ver9 (SAS, Cary, NC) and a medical statistician reviewed all data. Patients were divided into 3 groups according to Metavir score (F0-F1, F2-F3, F4). Data were expressed as mean ± standard deviation or median (interquartile range), appropriately. Comparison of PVSG, HVAT, HV-HA interval time, HV-PV interval time and serum albumin level was performed with the Turkey-Kramer test. Comparison of HASG, HVSG, ICG-R15, HA and PT% was performed non-parametrically with the Steel-Dwass test. Cirrhosis was defined as Metavir F4. ROC analyses to assess the diagnostic value of each parameter for cirrhosis

were conducted. The optimal cutoff value of each parameter was determined by the Youden index; that is, sensitivity + specificity -1 is maximized at the cutoff value. Spearman rank correlation coefficient analysis was used to test for correlations between the PVSG and conventional biochemical markers. The strength of the correlation was expressed as ρ. The ρ value was interpreted as follows: $0.7 \leq |\rho|$ = strong correlation; $0.4 \leq |\rho| < 0.7$ = moderate correlation, $0.2 \leq |\rho| < 0.4$ = weak correlation; $|\rho| < 0.2$ = no correlation between parameters. A two-sided p value < 0.05 was considered statistically significant.

[Results]

Sonazoid injection was well tolerated by all patients and no adverse events were noted. Studies were successfully performed in all patients.

Microbubble behavior of each vessel

In cases with normal livers, microbubbles first reached the HA, then the PV and finally the HV. The HA and PV were both strongly enhanced. In cirrhosis, microbubbles reached the HV earlier compared with the normal liver. Additionally, the visual intensity of the PV was weak (Fig. 4).

Slope gradient

The values of the SG of each vessel are shown in Table 2. The mean values of PV-SG were 29.1 ± 2.27 in the F0/1 group, 23.1 ± 1.86 in the F2/3 group and 14.7 ± 2.13 in the F4 group. There were significant differences among the groups (F0/1 group vs. F2/3 group, $p = 0.0476$; F0/1 group vs. F4 group, $p < 0.0001$; F2/3 group vs. F4 group, $p = 0.0044$). No significant differences were observed in HA-SG and HV-SG.

HVAT, HV-HA interval time and HV-PV interval time

The results of measurement of HVAT, HV-HA interval time and HV-PV interval time are shown in Table 2. The mean values of HVAT were 31.5 ± 1.81 seconds in the F0/1group, 23.4 ± 1.48 seconds in the F2/3 group and 27.3 ± 1.69 seconds in the F4 group. There were significant differences in the F0/1 group vs. the F2/3 group ($p = 0.0030$), but not between the F0/1 and F2/3 groups ($p = 0.2130$) or the F2/3 and F4 groups ($p = 0.2010$). The mean values of HV-HA interval time and HV-PV interval time were 10.5 ± 0.64 seconds and 6.45 ± 0.68 seconds, respectively, in the F0/1 group, 7.56 ± 0.52 seconds and 3.05 ± 0.56 seconds, respectively, in the F2/3 group, and 6.38 ± 0.60 seconds and 1.82 ± 0.64, respectively, in the F4 group. For both parameters, there were significant differences between the F0/1 and F2/3 groups ($p = 0.0025$, $p = 0.0010$, respectively) and the F0/1 and F4 groups ($p < 0.0001$, $p < 0.0001$, respectively), but not between the F2/3 and F4 groups ($p = 0.3037$, $p = 0.3202$, respectively).

Biochemical Markers

The levels of the conventional biochemical markers ICG-R15, HA, PT% and serum albumin level are shown in Table 2. The median ICG-R15 was 17.3% (12.3%-24.0%) in the F0/1 group, 22.2% (10.3%-37.5%) in the F2/3 group and 45.1% (30.8%-67.5%) in the F4 group. There were significant differences in the F0/1 group vs. the F4 group ($p = 0.0003$) and the F2/3 group vs. the F4 group ($p = 0.0083$) but not between the F0/1 vs. the F2/3 groups ($p = 0.4260$). The median HA was 43.5 ng/ml (23.8 ng/ml -66.3 ng/ml) in the F0/1 group, 124 ng/ml (62 ng/ml -266 ng/ml) in the F2/3 group and 465 ng/ml (238 ng/ml -863 ng/ml) in the F4 group. All the data showed significant differences between groups (F0/1 vs. F2/3, $p = 0.0023$; F0/1 vs. F4, $p < 0.0001$; F2/3 vs. F4, $p = 0.0083$). The median PT% was 98.5% (87.5%-103%) in the F0/1 group, 87.0% (82.5%-96.5%) in the F2/3 group and 71.5% (58.3%-94.0%) in the F4 group. Only the F0/1 group and the F4 group differed significantly ($p = 0.0088$). The mean level of serum albumin level was 4.03 ± 0.14 g/dl in the F0/1 group, 3.95 ± 0.11 g/dl in the F2/3 group and 3.50 ± 0.13 g/dl in the F4 group. There were significant differences in the F0/1 group vs. the F4 group ($p = 0.0176$) and the F2/3 group vs. the F4 group ($p = 0.0253$) but not between the F0/1 and the F2/3 groups ($p = 0.9081$).

Diagnostic accuracy

The diagnostic accuracy of PVSG, HVAT, HV-HA interval time, HV-PV interval time, ICG-R15, HA, PT% and serum albumin level for cirrhosis (Metavir = F4) was analyzed by ROC. The Area under the ROC (AUROC) for PVSG, HVAT, HV-HA interval time and HV-PV interval time was 0.83571, 0.54196, 0.74018 and 0.7623, respectively (Fig. 5). The AUROC for ICG-R15, HA, PT%, serum albumin level was 0.84196, 0.86161, 0.75000 and 0.78304, respectively (Fig. 5). Comparisons of the AUROCs for PVSG and other parameters are shown in Table 3. The AUROC of PVSG was statistically different compared with that of HVAT but no differences were observed in comparisons with other parameters (Table 3).

The optimal cutoff value of each parameter was determined by the Youden Index (Table 4). The PVSG showed a sensitivity of 62.5%, a specificity of 94.3%, and an accuracy of 86.3%.

Correlations between the PVSG and biochemical markers

Scatter diagrams and correlation analyses of PVSG and ICG-R15, HA, PT% and serum albumin level were shown in Figure 6 and Table 5. ICG-R15 and HA showed moderate correlation with PVSG with statistical differences ($\rho = -0.5691$, $p < 0.0001$ for ICG-R15, $\rho = -0.4652$, $p = 0.0006$ for HA). PT% and serum albumin level showed weak correlation with the PVSG with statistical differences ($\rho = 0.3015$, $p = 0.0315$ for PT%, $\rho = 0.3769$, $p = 0.0064$ for serum albumin level).

[Discussion]

In this study, we evaluated a new modality to diagnose cirrhosis and compared it with conventional parameters. We observed that the PVSG of TIC in patients with compensated cirrhosis was significantly lower than that in non-cirrhotic patients. When a PVSG cut off value was determined at 15 for the diagnosis of cirrhosis, the specificity and accuracy were as high as 94.3% and 86.3%. To our knowledge, this is the first report to demonstrate that PVSG measurement with an ultrasound microbubble contrast agent discriminates cirrhosis from non-cirrhosis. All of the patients in our study were candidates for surgical treatment. The patients in the F4 group had well-compensated cirrhosis, not advanced cirrhosis. In the diagnosis of well-compensated cirrhosis, the PVSG, which is calculated by the combination of the signal intensity of the PV and the transient time of the contrast agent in the PV made the accuracy higher than the transient time analyses only.

Previous studies have shown that the HVAT, HV-HA interval, and HV-PV interval have high accuracy in the diagnosis of cirrhosis and can be used to predict disease severity [8, 12]. These measurements reveal intrahepatic arterio-venous and

porto-venous shunting caused by vascular remodeling at the sinusoidal level. However, in our study, the diagnostic accuracy of these parameters for the diagnosis of cirrhosis was lower than we expected (Table 4) [8, 12]. One possible reason is that the contrast agent injection time may vary among patients. In this study, three different collaborators injected the contrast agent manually, with likely variation among injection times, ultimately affecting the HVAT. HV-HA and HV-PV interval times were more accurate than HVAT as they were not affected by individual variations in injection times. Although, in our results, the mean HV-HA interval time and HV-PV interval time were shorter than those reported [12]. The transit time of the contrast agent is reported to become shorter in patients with liver tumors because of tumoral arterio-venous or porto-venous shunting [18, 19, 20]. All of the patients in our study had liver tumors, which may have made transient times shorter, affecting the accuracy of a diagnosis of cirrhosis. Concerning this, in the clinical setting, many patients with cirrhosis have liver tumors, and the ability to detect well-compensated cirrhosis in these patients is critical for surgical candidates. In this respect, the PVSG, which is not affected by tumoral intrahepatic shunting, is thought to be more useful than transient time

analyses.

As for the intrahepatic arterial blood flow, it is well known that hepatic arterial flow increases in patients with liver cirrhosis, to compensate for the decreased PV blood flow by the “hepatic arterial buffer response” [14]. Despite this, our results revealed no significant difference in HASG between the F4 group and the other groups. In the report of Doppler sonography, a high resistive index of the HA is seen in patients with severe cirrhosis, but HA flow remains normal in the most of the cirrhotic patients [21, 22]. The subjects in this study were limited to well-compensated cirrhosis, and our results showed that HA flow was not dramatically changed in this group of patients.

To assess the clinical significance of the PVSG, we compared the AUROC of the PVSG with other diagnostic parameters. The AUROC of the PVSG was higher than that of the HVAT and interval times. Compared with the conventional biochemical parameters, the AUROCs of the ICG-R15 and HA were higher than that of the PVSG. As a result, the diagnostic impact of the PVSG was not superior to those of the ICG-R15 and HA. Despite this, the PVSG had high specificity and accuracy in diagnosing cirrhosis (94.3% and 86.3%, respectively). Also in the correlation analysis, the PVSG showed moderate

correlations with ICG-R15 and HA. There are many patients in whom it is difficult to distinguish between cirrhosis and non-cirrhosis with one parameter, especially patients with well-compensated cirrhosis. Our results emphasize that combination assays including the PVSG and other parameters including ICG-15R and HA could predict well-compensated cirrhosis more accurately.

There are some limitations to this study. First, it was cross-sectional and involved different etiologies of liver disease, including HBV, HCV, alcoholic, and cryptogenic hepatitis. From the pathologic standpoint, major differences have been reported between cirrhosis caused by hepatitis viruses and that caused by alcoholism, with a resultant difference in intrahepatic hemodynamics [23]. The smaller regenerative nodules in alcoholic cirrhosis are more likely to cause venous compression and to impede early outflow, leading to portal hypertension. Furthermore, in cases of viral hepatitis, it is reported that certain histologic characteristics of HCV cirrhosis are distinct from those of HBV cirrhosis. Therefore, a future project could be composed of a cohort recruited from a homogeneous group of patients. A second limitation is that different microbubble contrast agents were used in prior studies on which we based our

comparisons. The majority of reported studies were performed with Levovist (Schering, Berlin, Germany), while other studies were performed with Optison (Amersham Health, Milwaukee, WI, USA) [24], SonoVue (Bracco, Milan, Italy) [25] and Sonazoid (GE Healthcare, Oslo, Norway) [16]. These agents have different chemical properties. Levovist, a first-generation agent, is very fragile against acoustic pressure, and SonoVue and Sonazoid, second-generation agents, are more stable. Bloomly et al [26] showed that Levovist and Sonazoid are taken up in the liver and spleen beyond the vascular phase, while Lim et al [25, 27] demonstrated definite uptake of SonoVue in the spleen but no substantial uptake in the liver. These differences could possibly lead to different signal intensities and a transit times. Consideration of these limits is important in functional examinations with microbubble contrast agent, and it should be clarified in the further studies.

In conclusion, we have shown for the first time that the PVSG is a unique and reliable test that has the potential to be a diagnostic tool to identify well-compensated cirrhosis for surgical candidates in combination with other conventional modalities. Although this technique needs further investigations, it is promising as a useful tool for the

management of patients with chronic liver disease and for the preoperative assessment of cirrhosis.

Conflict of interest statement: Yuichi Goto and each of the co-authors have no conflict of interest.

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[Legends]

Fig. 1. The intensity of each vessel was measured by setting circular ROIs in each vessel with ImageJ. Arrow is the ROI for the HA, broken arrow is the ROI for the PV and arrowhead is the ROI for the HV. The ROIs were set in the vessels at a depth of 6-10 cm depth (± 2 cm from the focus point) from the surface.

Fig. 2. Time intensity curves of each vessel in a normal liver (a) and in a cirrhotic liver (b) are shown. Red line is the signal intensity of the HA, green line is the signal intensity of the PV and blue line is the signal intensity of the HV. In cirrhosis, the slope of the PV is gentle as compared with that of the normal liver.

Fig. 3. An example of the slope gradient of the portal vein (PV) is shown. The PV slope gradient (PVSG) was obtained by the linear approximation method between the arrival time (a) and the peak enhancement time (b). In the figure, the PVSG is 16.7.

Fig. 4. Cases of pulse-inversion imaging in the normal liver (a-d) and cirrhotic liver (e-h) are shown. In a normal liver, the contrast agent arrives first in the HA (a; arrow), then

in the PV (b; arrow) and finally in the HV (d; black arrow). The HA and the PV are both strongly enhanced (b, c). In cirrhosis, microbubbles reach in the HV (g; arrow) earlier than in the normal liver. The intensity of the portal vein (h; arrow) is weak compared with normal liver.

Fig. 5. ROC of the PVSG (a), the HVAT (b), the HV-HA interval time (c), the HV-PV interval time (d), ICG-R15 (e), Hyaluronic acid (f), Prothrombin time (g), and Albumin (h) for the diagnosis of cirrhosis (Metavir = F4).

Fig. 6. Scatter diagram of the PVSG and ICG-R15, Hyaluronic acid, Prothrombin time and Albumin.

Table 1. Characteristics of patients and liver tumors in each group

Values are mean ± standard deviation.

Table 2. Values of the slope gradient of the hepatic vessels, HVAT, interval times and conventional biochemical markers in each group

HASG, hepatic artery slope gradient; PVSG, portal vein slope gradient; HVSG, hepatic vein slope gradient; HVAT, hepatic vein arrival time; HV, hepatic vein; HA, hepatic artery; PV, portal vein. Values are mean ± standard deviation or median (interquartile range).

Table 3. AUROC of each parameters and comparison of the AUROC between the PVSG and biochemical parameters and CEUS parameters.

AUROC, area under the receiver operating characteristic; PVSG, portal vein slope gradient; CEUS, contrast-enhanced ultrasonography; HVAT, hepatic vein arrival time; HV, hepatic vein; HA, hepatic artery; CI, confidence interval.

Table 4. Sensitivity, specificity, and accuracy of the PVSG, HVAT, interval times and

conventional biochemical markers for the diagnosis of cirrhosis

PVSG, portal vein slope gradient; HVAT, hepatic vein arrival time; HV, hepatic vein; HA, hepatic artery; PV, portal vein.

Table. 5. Correlation between the PVSG and conventional biochemical markers.

PVSG, portal vein slope gradient.

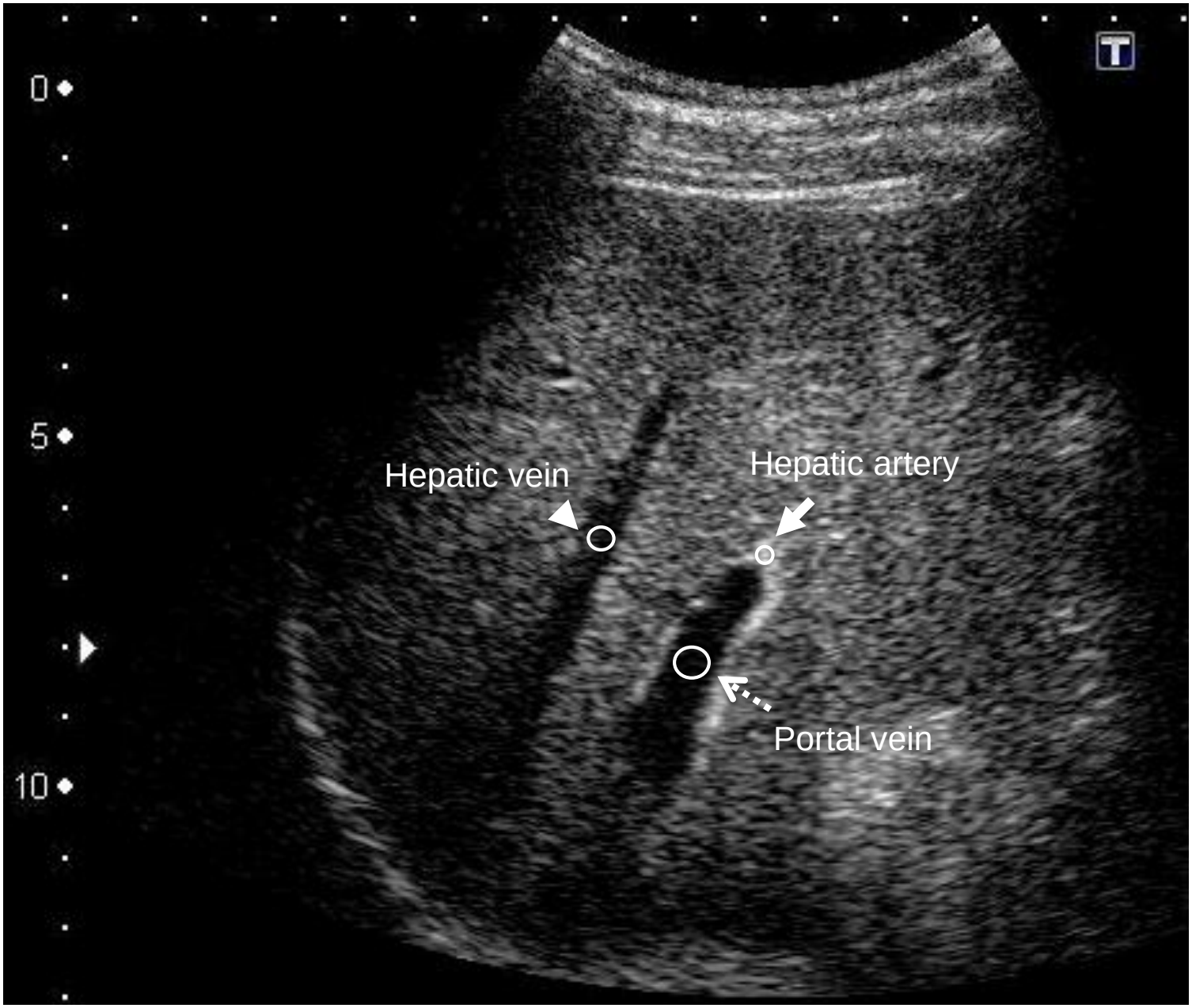

Fig. 1.

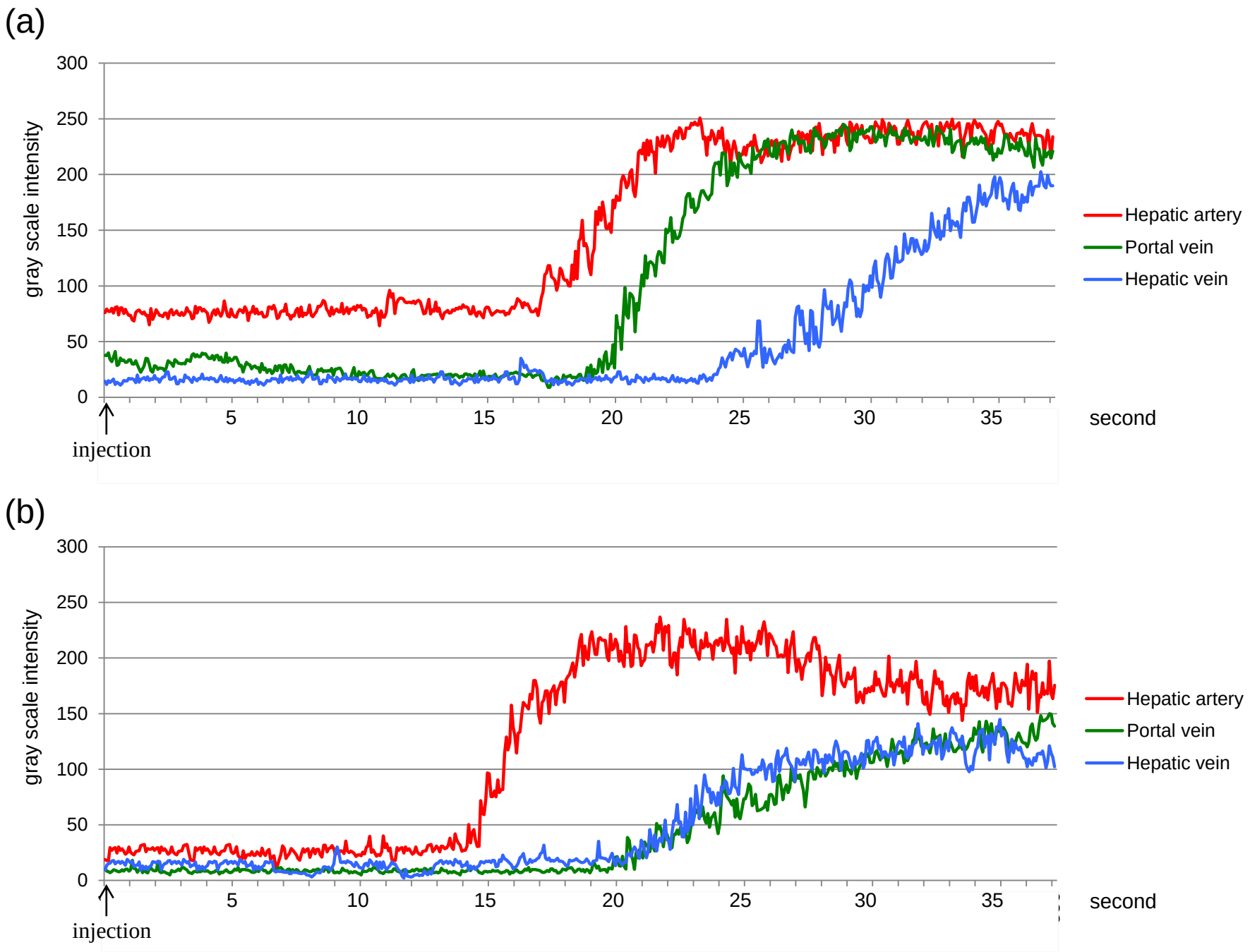
(a)
gray scale intensity
300
250
200
150
100
50
0
5
10
15
20
25
30
35
second
injection
Hepatic artery
Portal vein
Hepatic vein
(b)
gray scale intensity
300
250
200
150
100
50
0
5
10
15
20
25
30
35
second
injection
Hepatic artery
Portal vein
Hepatic vein

Fig. 2.

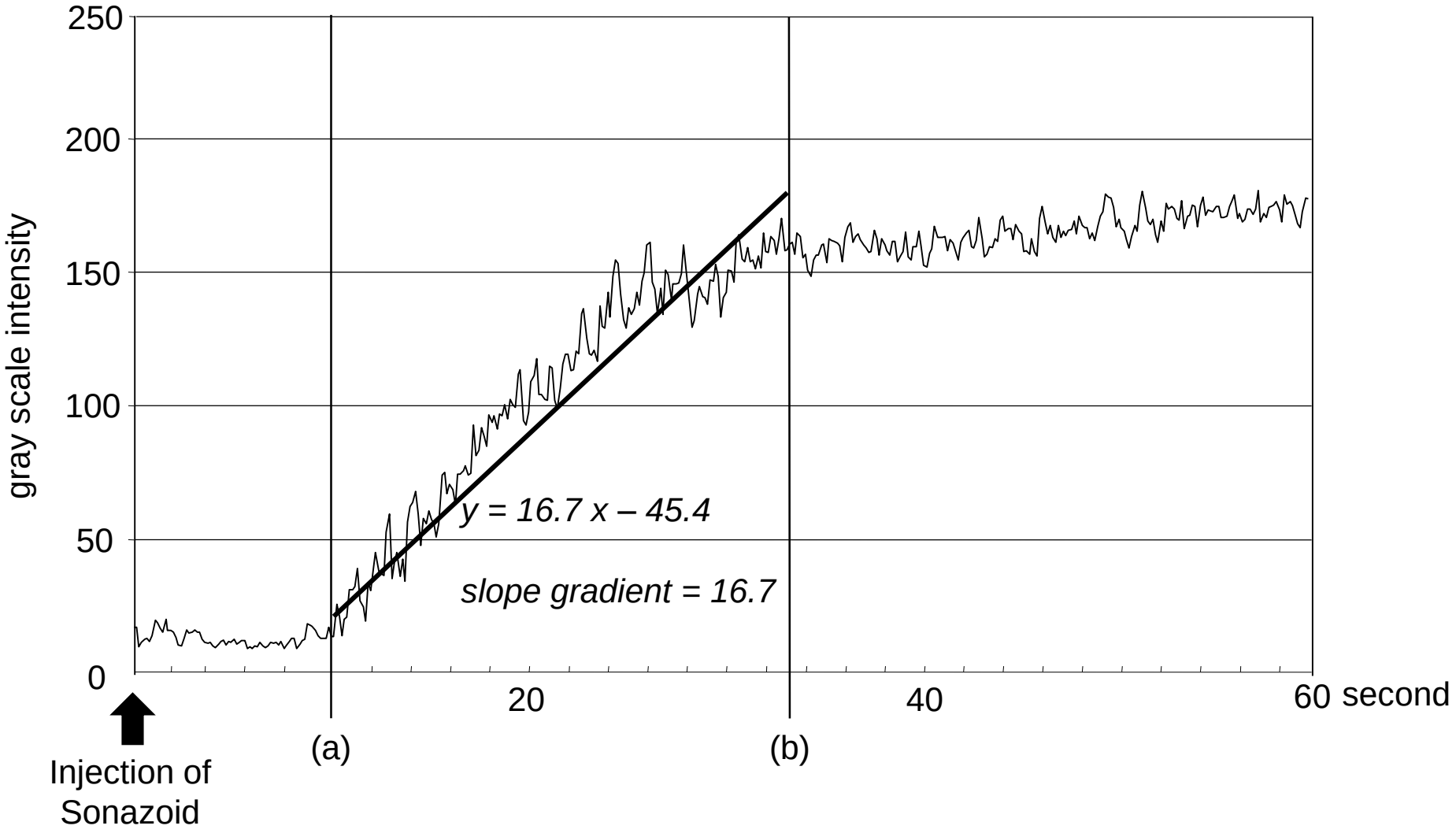
250
200
150
100
50
0
gray scale intensity
y = 16.7 x – 45.4
slope gradient = 16.7
20
40
60 second
(a)
(b)
Injection of
Sonazoid

Fig. 3.

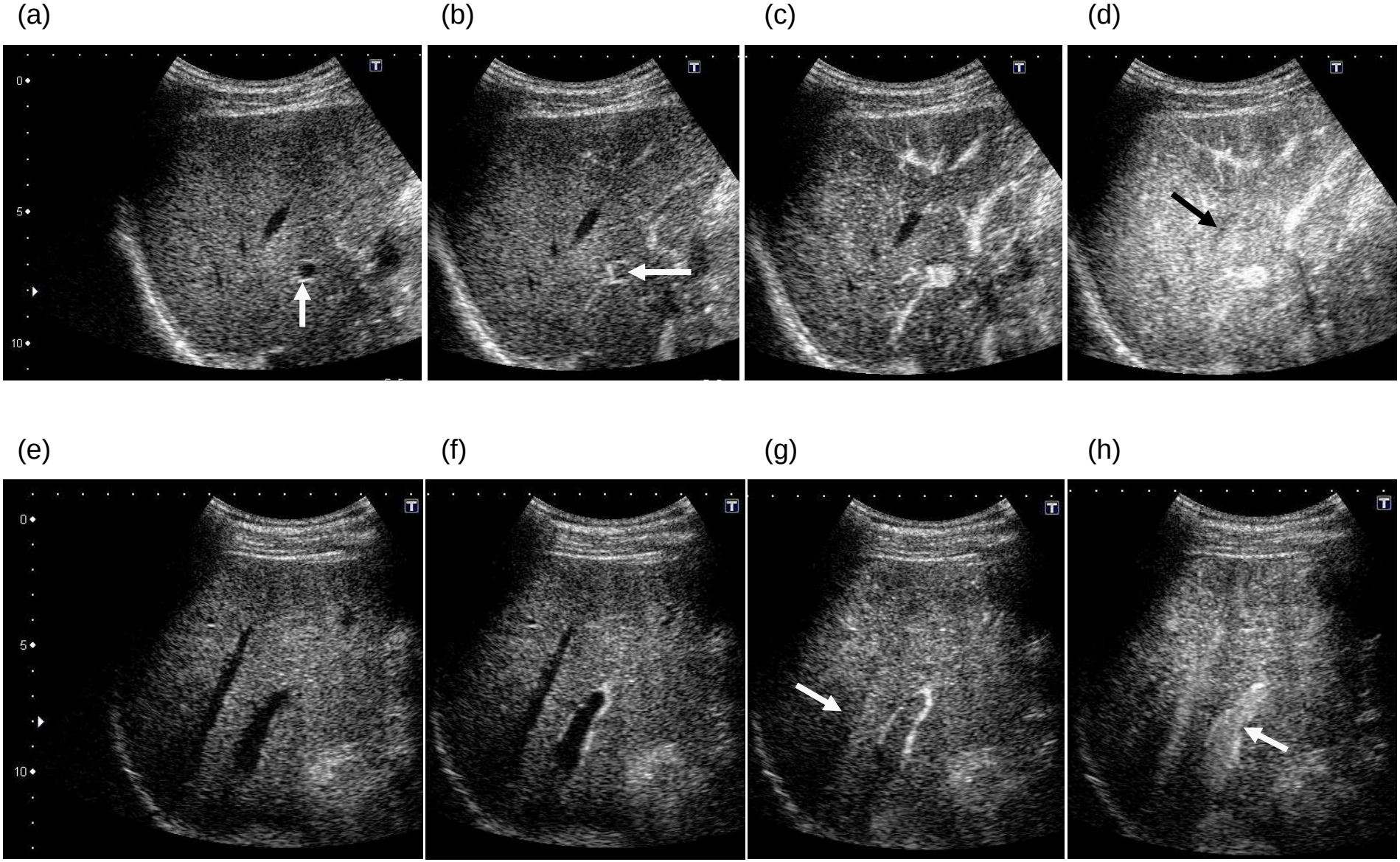

Fig. 4.

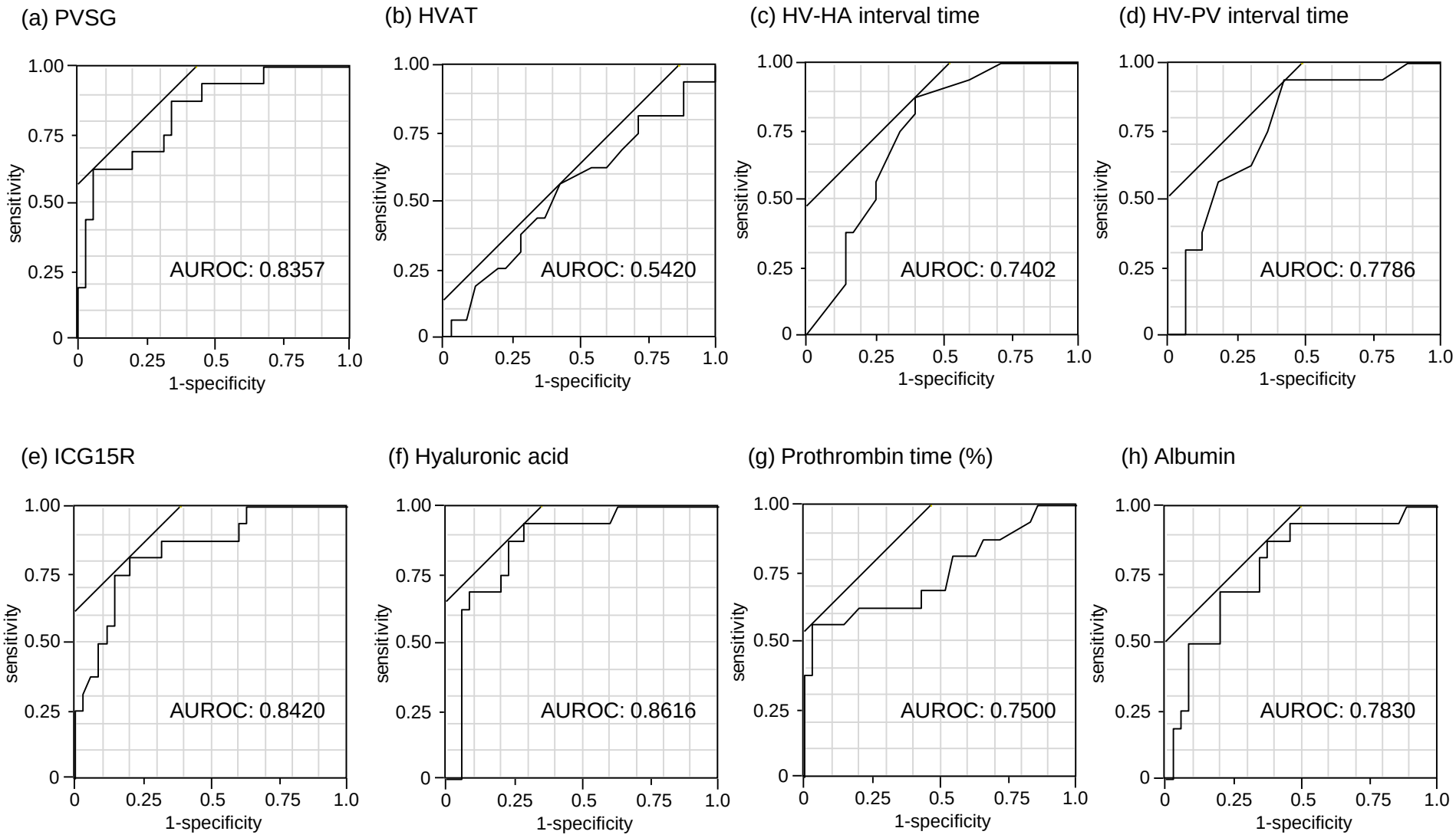
(a) PVSG
AUROC: 0.8357
(b) HVAT
AUROC: 0.5420
(c) HV-HA interval time
AUROC: 0.7402
(d) HV-PV interval time
AUROC: 0.7786
(e) ICG15R
AUROC: 0.8420
(f) Hyaluronic acid
AUROC: 0.8616
(g) Prothrombin time (%)
AUROC: 0.7500
(h) Albumin
AUROC: 0.7830
sensitivity
1-specificity
0
0.25
0.50
0.75
1.00

Fig. 5.

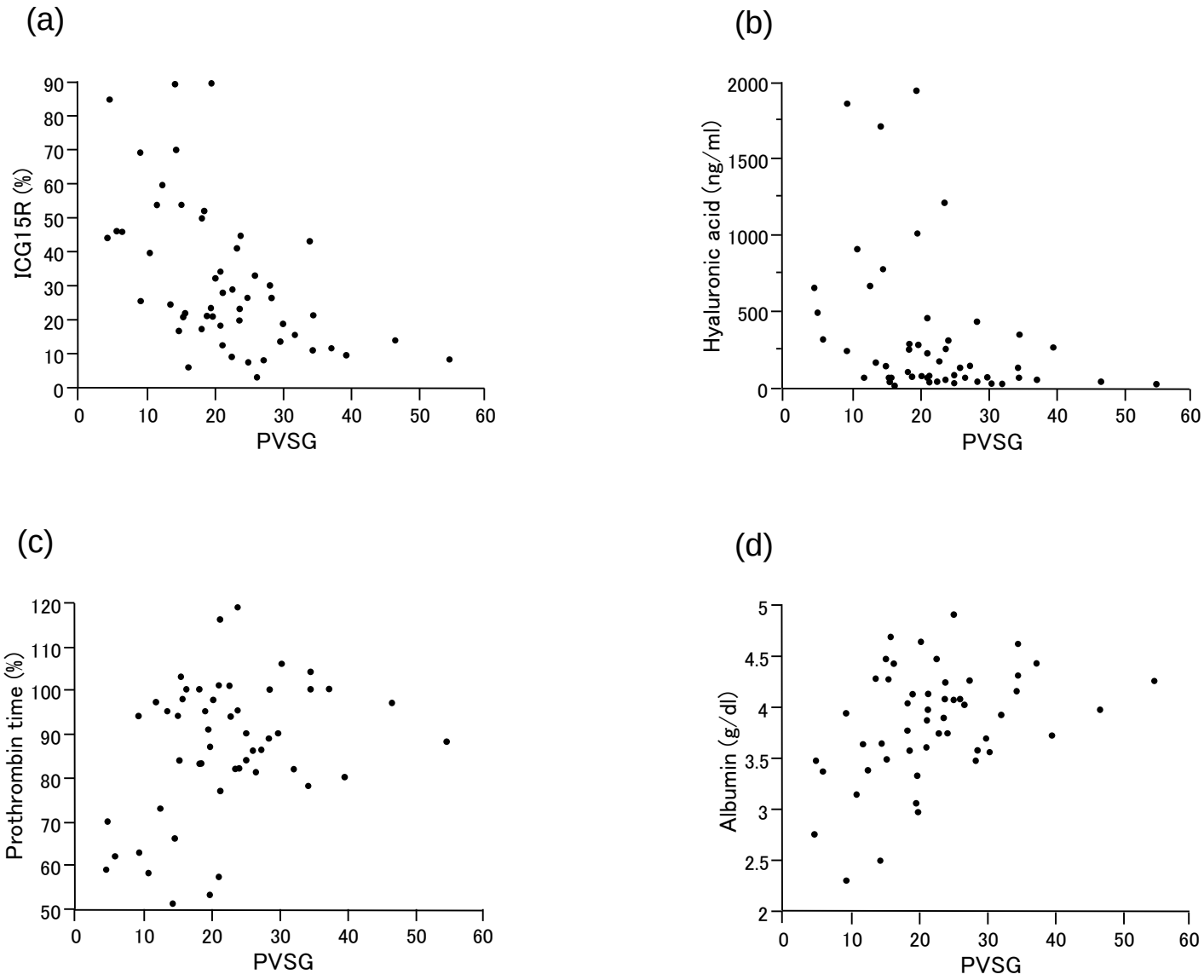
(a)
ICG15R (%)
PVSG
(b)
Hyaluronic acid (ng/ml)
PVSG
(c)
Prothrombin time (%)
PVSG
(d)
Albumin (g/dl)
PVSG

Fig. 6.

Table 1. Characteristics of patients and liver tumors in each group

	F0/1 group n = 14	F2/3 group n = 21	F4 group n = 16
Age (years)	69.6 ± 2.97	65.0 ± 2.42	67.4 ± 2.77
Male/Female	10/4	15/6	10/6
Body mass index (kg/m^2)	21.9 ± 0.62	22.2 ± 0.51	22.7 ± 0.58
HCV/ HBV/ HCV+HBV/ Alcoholic/ Cryptogenic	1/ 0/ 0/ 0/ 0	14/ 2/ 1/ 2/ 2	13/ 1/ 0/ 2/ 0
Child-Pugh grade A / B			16 / 0
Tumor size (mm)	24.5 ± 2.20	27.3 ± 1.80	19.7 ± 2.06
Tumor number	1.29 ± 0.12	1.24 ± 0.10	1.31 ± 0.11
AST (IU/L)	24.6 ± 5.33	50.4 ± 4.35	58.3 ± 4.99
ALT (IU/L)	18.7 ± 5.99	47.8 ± 4.89	44.3 ± 5.60
Total bilirubin (mg/dl)	0.77 ± 0.15	0.91 ± 0.12	1.37 ± 0.14
Platelet count ($\times 10^4$)	17.5 ± 1.08	11.9 ± 0.88	10.9 ± 1.01
Prothrombin time (% of normal)	97.1 ± 3.60	88.4 ± 2.93	75.4 ± 3.37
Albumin (g/dl)	4.03 ± 0.14	3.96 ± 0.11	3.50 ± 0.13

Values are mean ± standard deviation.

Table 2. Values of slope gradient of the hepatic vessels, HVAT, interval times and conventional biochemical markers in each group

	F0/1 group n = 14	F2/3 group n = 21	F4 group n = 16	p value		
				F0/1 vs. F2/3	F0/1 vs. F4	F2/3 vs. F4
Slope Gradients						
HASG	24.7 (10.9-49.1)	22.4 (15.4-29.8)	20.2 (18.2-28.9)	0.8968	0.7835	0.8968
PVSG	29.1 ± 2.27	23.1 ± 1.86	14.7 ± 2.13	0.0476	<0.0001	0.0044
HVSG	10.0 (6.08-12.1)	13.6 (5.67-22.9)	12.3 (9.59-24.0)	0.4261	0.5390	0.8533
HVAT and Interval Times						
HVAT (sec)	31.5 ± 1.81	23.4 ± 1.48	27.3 ± 1.69	0.0030	0.2130	0.2010
HV-HA interval time (sec)	10.5 ± 0.64	7.56 ± 0.52	6.38 ± 0.60	0.0025	<0.0001	0.3037
HV-PV interval time (sec)	6.45 ± 0.68	3.05 ± 0.56	1.82 ± 0.64	0.0010	<0.0001	0.3202
Biochemical Markers						
ICG15R (%)	17.3 (12.3-24.0)	22.2 (10.3-37.5)	45.1 (30.8-67.5)	0.4260	0.0003	0.0083
Hyaluronic acid (ng/ml)	43.5 (23.8-66.3)	124 (62.0-266)	465 (238-863)	0.0023	<0.0001	0.0083
Prothrombin time (%)	98.5 (87.5-103)	87.0 (82.5-96.5)	71.5 (58.3-94.0)	0.1107	0.0088	0.0927
Albumin (g/dl)	4.03 ± 0.14	3.95 ± 0.11	3.50 ± 0.13	0.9081	0.0176	0.0253

HASG, hepatic artery slope gradient; PVSG, portal vein slope gradient; HVSG, hepatic vein slope gradient; HVAT, hepatic vein arrival time; HV, hepatic vein; HA, hepatic artery; PV, portal vein. Values are mean ± standard deviation or median (interquartile range).

Table 3. AUROC of each parameter and comparison of the AUROC between the PVSG and biochemical parameters and CEUS parameters

Parameters	AUROC	p vs. PVSG	95% CI
PVSG	0.8357	-	0.6805-0.9240
HVAT	0.5420	0.0039	0.3662-0.7079
HV-HA interval time	0.7402	0.3278	0.5860-0.8515
HV-PV interval time	0.7786	0.5248	0.6062-0.8788
ICG15R	0.8420	0.9319	0.6877-0.9280
Hyaluronic acid	0.8616	0.7441	0.7142-0.9394
Prothrombin time (%)	0.7500	0.5551	0.5551-0.8782
Albumin	0.7830	0.6159	0.6159-0.8904

AUROC, area under the receiver operating characteristic; PVSG, portal vein slope gradient; CEUS, contrast-enhanced ultrasonography; HVAT, hepatic vein arrival time; HV, hepatic vein; HA, hepatic artery; CI, confidence interval.

Table 4. Sensitivity, specificity, and accuracy of the PVSG, HVAT, interval times and conventional biochemical markers for the diagnosis of cirrhosis

	Cutoff value	Sensitivity (%)	Specificity (%)	Accuracy (%)
PVSG	< 15	62.5	94.3	86.3
HVAT (sec)	< 28	56.3	57.1	56.8
HV-HA interval time (sec)	< 8.4	87.5	60	68.6
HV-PV interval time (sec)	< 4	93.8	57.1	68.6
ICG15R (%)	> 30	81.3	80	80.4
Hyaluronic acid (ng/ml)	> 131	93.8	71.4	78.4
Prothrombin time (%)	< 73	56.2	97.1	84.3
Albumin (g/dl)	< 3.93	87.5	62.9	70.6

PVSG, portal vein slope gradient; HVAT, hepatic vein arrival time; HV, hepatic vein; HA, hepatic vein; PV, portal vein.

Table 5. Correlations between the PVSG and conventional biochemical markers.

Parameters	ρ	p value
ICG15R	-0.5691	<.0001
Hyaluronic acid	-0.4652	0.0006
Prothrombin time (%)	0.3015	0.0315
Albumin	0.3769	0.0064

PVSG, portal vein slope gradient.