ORIGINAL ARTICLE

Measuring Intrahepatic Vascular Changes Using Contrast-Enhanced Ultrasonography to Predict the Prognosis of Alcoholic Hepatitis Combined with Cirrhosis: A Prospective Pilot Study

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Background/Aims: Acute hepatic dysfunction combined with alcoholic hepatitis (AH) in alcoholic cirrhosis is related to hepatic hypo-perfusion secondary to intrahepatic necro-inflammation, neoangiogenesis, and shunt. The hepatic vein arrival time (HVAT) assessed by microbubble contrast-enhanced ultrasonography (CEUS) is closely correlated with the severity of intrahepatic changes. We investigated the usefulness of HVAT to predict short-term mortality of AH in cirrhosis. **Methods:** Thirty-nine patients with alcoholic cirrhosis (27 males) and AH were prospectively enrolled. HVAT study was performed within 3 days after admission using ultrasonic contrast (SonoVue®). The primary outcome was 12-week mortality. **Results:** Twelve-week mortality developed in nine patients. HVAT was significantly different between the mortality and survival groups (9.3±2.0 seconds vs 12.6±3.5 seconds, p=0.002). The odds ratio of a shortened HVAT for 12-week mortality was 1.481 (95% confidence interval, 1.050–2.090; p=0.025). The area under the receiver operating characteristic curve of HVAT for 12-week mortality was 0.787 (p=0.010). The combination of MDF and HVAT ≥11.0 seconds resulted in an 87.5% survival rate even if the MDF score ≥32; however, HVAT <11.0 seconds was related with mortality despite a MDF score<32. **Conclusions:** HVAT using microbubble CEUS could be a useful additional index to predict short-term mortality in patients with AH and cirrhosis. **(Gut Liver 2018;12:555-561)**

Key Words: Hepatitis, alcoholic; Liver cirrhosis, alcoholic; Hepatic veins; Ultrasonography, contrast-enhanced

INTRODUCTION

Alcoholic liver disease is one of leading cause of chronic liver disease and progression to end-stage liver disease.[1] Alcoholic hepatitis (AH) is a phenotype of alcoholic liver disease and severe cases show high short-term mortality of 40% to 50%.[2,3] Cirrhosis is a pathological diagnosis characterized by diffuse fibrosis, severe disruption of the intrahepatic vascular system with neoangiogenesis and formation of a shunt resulting in portal hypertension and liver failure.[4,5] Acute hepatic dysfunction combined with AH caused by binge drinking in patients with alcoholic cirrhosis is a common cause of acute on chronic liver failure and a hyper-acute poor prognosis.[6] The poor prognosis is closely related to intrahepatic histological changes, including acute necroinflammation, secondary intrahepatic vascular disarrangement, and hepatic hypo-perfusion.

Several scoring systems have been used to predict survival of patients with AH, including Maddrey's Discriminant Function (MDF) score,[7] the Model for End-Stage Liver Disease (MELD) score,[8] the Glasgow Alcoholic Hepatitis score,[9] age, serum bilirubin, international normalized ratio of prothrombin time and serum creatinine,[10] the hepatic venous pressure gradient[11] and the Lille model.[12] MDF is one of the most commonly used systems and when MDF is ≥32 it is generally defined as severe alcoholic hepatitis (SAH). The treatment outcome of AH using prednisolone or pentoxifylline is relatively poor and is actually a clinically urgent situation that requires an early decision for liver transplantation (LT) before or regardless of starting treatment.[13] These scoring systems have limitations because they include some serological components that do not properly represent the

rapid changes in disease severity and, particularly, the acute intrahepatic histological vascular changes in the early stage.

The hepatic vein arrival time (HVAT), as assessed by microbubble contrast-enhanced ultrasonography (CEUS), is the time (in second) taken for microbubble contrast agent to arrive at the hepatic vein (HV) after intravenous injection. It is a safe noninvasive method to estimate the severity and intrahepatic histological changes, particularly intrahepatic vascular changes.[14-16] Therefore, we investigated the possibility of using HVAT as an additional prognostic index representing immediate intrahepatic histological changes to predict short-term mortality of patients with cirrhotic AH.

MATERIALS AND METHODS

1. Study population

Patients between 20 and 70 years of age with underlying cirrhosis and AH related with binge drinking alcohol who visited the Wonju Severance Christian Hospital, Yonsei University Wonju College of Medicine from March 2015 to February 2016 were considered eligible for this prospective study. The inclusion criteria were: (1) excessive alcohol consumption within 2 months (>60 g/day for males and 40 g/day for females); (2) rapid deterioration of liver function during the past 2 months; and (3) aspartate/alanine aminotransferase (AST/ALT) ratio greater than 2 with an AST level >40 IU/L. Exclusion criteria were: (1) those with liver disease not related to alcohol consumption (i.e., viral hepatitis, autoimmune hepatitis, and drug-induced liver injury) or hepatocellular carcinoma; and (2) those receiving pentoxifylline and/or corticosteroids before baseline HVAT study was performed. (3) In addition, patients who have already combined with infection and sepsis were also excluded. Patients who did not provide informed consent to participate were also excluded from the study. Thus, among 89 patients who were included in the initial screening, 50 patients were excluded, and

39 patients were finally enrolled and completed the laboratory tests, HVAT measurements, and a 12-week follow-up with appropriate medical therapies including steroid and pentoxifylline (Fig. 1).

2. Study design and endpoint

Baseline clinical data, Child-Pugh score, MDF, MELD, and HVAT were measured simultaneously on the same day within 3 days of admission. MDF was calculated with the equation: MDF=(4.6×[patient's prothrombin time in second–control's prothrombin time in second]+patient's serum bilirubin level [mg/dL]). The MELD score was also calculated using an automatic calculating program at a website. After enrollment, the patients were followed up with or without special treatment, such as glucocorticoid or pentoxifylline, depending on the clinical indication. The primary endpoint was liver-related 12-week mortality, and the predictive values of each clinical scoring systems were evaluated.

This prospective study was approved by the Institutional Review Board of Wonju Severance Hospital (IRB No. CR211016) and performed in accordance with the principles of the Declaration of Helsinki. The informed consents were obtained.

3. Measurement of hepatic vein arrival time

All enrolled patients underwent measurement of HVAT using CEUS within 3 days of admission. After an overnight fast, all CEUS procedures were performed by an examiner (M.Y.K.) using a 2.5-μm (range, 2 to 8 μm) second-generation sulfur hexafluoride microbubble-based contrast agent (SonoVue®; Bracco SpA, Milan, Italy) according to a method described previously.[15] The agent was injected into an antecubital vein, and signals were recorded from the right or middle hepatic veins for analysis. HVAT was calculated as the time interval from injection to the second point on the curve representing the signal intensity that exceeded the baseline intensity by 10% (Fig. 2). The HVAT cut-
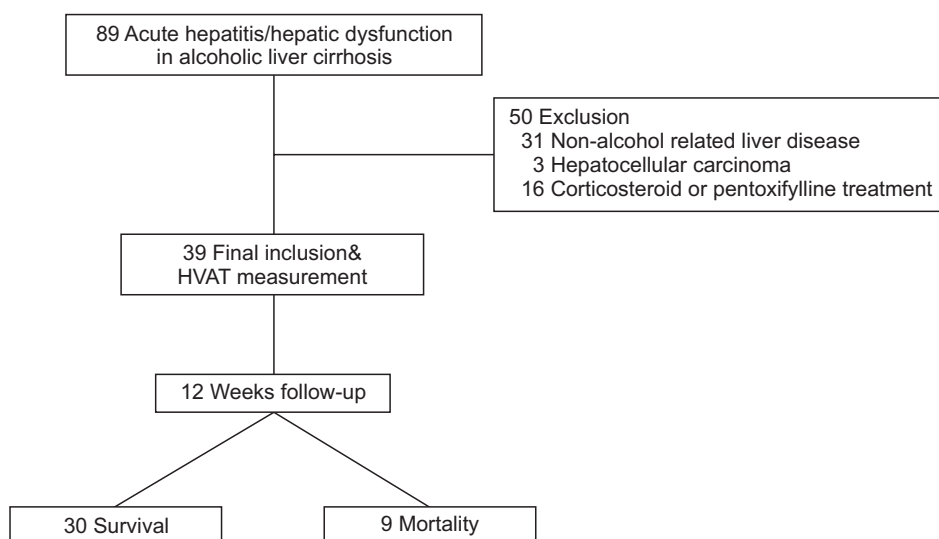

Fig. 1. Study design and flow chart HVAT, hepatic vein arrival time.

off value (11.0 seconds) was determined according to Youden index based on receiver operating characteristic (ROC) curve analysis.

4. Statistical analysis

Continuous variables are presented as mean±standard deviation. Categorical variables are presented as counts and propor-

tions. Group comparisons of continuous variables were conducted with the independent t-test and the Mann-Whitney U-test, as appropriate. Categorical variables were compared with the chi-square test. Pearson's correlation analysis was used for normally distributed variables and Spearman's rank-correlation coefficient analysis was performed for non-normally distributed data, as appropriate. To assess the performance of HVAT in pre-
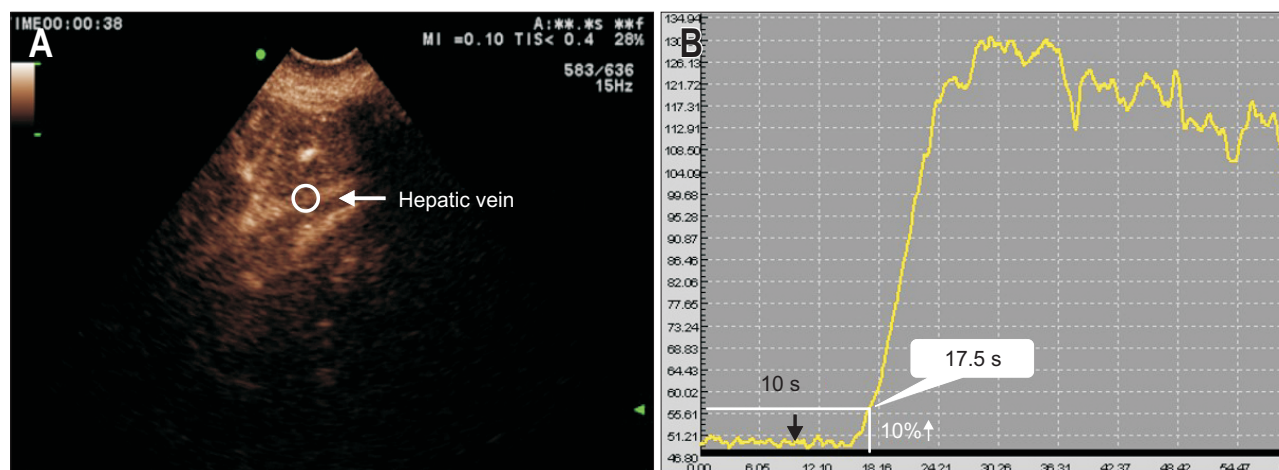

Fig. 2. Hepatic vein arrival time (HVAT) measurement using microbubble contrast enhanced ultrasonography (CEUS). (A) Detection of hepatic vein (HV) enhancement. After contrast injection at 10 seconds of lead time, HV enhancement with microbubble contrast agent was detected (white circle is region of interest [ROI] to measure time intensity curves [TICs]). (B) TICs for HV enhancement intensity were drawn and HVAT was calculated as the time (in seconds) from injection to a sustained signal increase in the TIC to over 10% above baseline intensity point (the 10-second lead time [black arrow] should be subtracted from the measured time point [e.g., 17.5–10.0 seconds=7.5 seconds]).

Table 1. General Characteristics

Characteristic	Total population (n=39)	Survival (n=30)	Mortality (n=9)	p-value*
Male sex	27 (69.0)	22 (73.3)	5 (55.6)	0.416
Age, yr	50.0 (35.0–69.0)	51.0 (35.0–68.0)	46.0 (42.0–69.0)	0.921
Child-Pugh score	9.0 (8.0 –11.0)	9.0 (5.0–10.0)	10.0 (7.0–13.0)	0.007
MELD score	19.5 (6.0–40.0)	16.0 (6.0–28.0)	27.0 (7.0–40.0)	<0.001
MDF score	49.0 (1.6–113.9)	32.2 (1.6–80.0)	78.2 (58.4–113.9)	<0.001
HVAT, s	11.0 (6.4–19.4)	12.2 (7.1–19.4)	8.7 (6.4–16.8)	0.002
Total bilirubin, mg/dL	8.7 (0.8–54.6)	4.8 (0.8–28.9)	21.9 (1.1–54.6)	0.001
AST, U/L	165.5 (50.0–2,185.0)	296.0 (50.0–2,185.0)	150.0 (61.0–300.0)	0.005
ALT, U/L	85.5 (17.0–2,635.0)	256.0 (20.0–2,635.0)	37.0 (17.0–252.0)	0.005
Albumin, g/dL	3.1 (2.0–4.4)	3.3 (2.2–4.4)	3.0 (2.0–3.6)	0.175
INR	1.5 (0.9–2.7)	1.4 (0.9–2.3)	2.0 (1.0–2.7)	<0.001
WBC, 10^9/L	5,765.0 (1,730.0–32,490.0)	5,500.0 (1,730–32,490)	6,750.0 (4,150.0–19,000.0)	0.018
Hb, g/dL	11.8 (8.3–17.3)	12.1 (8.5–17.3)	11.0 (8.3–14.4)	0.130
Platelet, 10^9/L	106.0 (25.0–443.0)	110.0 (25.0–443.0)	80.0 (25.0–295.0)	0.817
CRP, mg/dL	0.9 (0.1–7.8)	0.6 (0.1–7.8)	2.0 (0.3–4.4)	0.173
Cr, mg/dL	0.7 (0.2–3.5)	0.6 (0.2–1.5)	0.7 (0.2–3.5)	0.305
Na, mmol/L	137.0 (117.0–144.0)	138.0 (117.0–144.0)	137.0 (125.0–139.0)	0.360

Data are presented as number (%) or median (range).
MELD, Model for End-Stage Liver Disease; MDF, Maddrey's Discriminant Function; HVAT, hepatic vein arrival time; AST, aspartate aminotransferase; ALT, alanine aminotransferase; INR, international normalized ratio; WBC, white blood cell; Hb, hemoglobin; CRP, C-reactive protein.
*Statistical comparison between survival and mortality.

dicting mortality, ROC curves and the area under the ROC curve (AUROC) were calculated. Odds ratios of the factors predicting 12-week mortality were calculated using binary logistic regression analysis. A p-value <0.05 was considered significant. The statistical analysis was performed with IBM SPSS version 20.0 software (IBM Corp., Armonk, NY, USA).

RESULTS

The baseline general characteristics of study populations are summarized in Table 1. Among the 39 included patients, nine patients died within 12 weeks. The causes of death were gastrointestinal bleeding (one patient), spontaneous bacterial peritonitis (one patient), sepsis (two patients), hepatic failure (five patients). As expected, the indices suggesting hepatic dysfunction, such as the Child-Pugh score, MDF, and MELD score, were higher and HVAT was significantly shorter in the mortality group. Leukocytosis was also prominent in the mortality group compared to the surviving group (Table 1).

1. Traditional predictive scoring systems related to mortality

Significant relationships were observed between mortality and the clinical scores. The increases in the Child-Pugh, the MELD, and MDF scores indicated an increased risk for 12-week mortality (odds ratios=2.469, 1.434, and 1.071 respectively) (Table 2). The AUROC values for 12-week mortality with the Child-Pugh score, MELD score, and MDF score were 0.804 (p=0.006), 0.915 (p<0.001), and 0.915 (p<0.001), respectively.

2. Hepatic vein arrival time and mortality

HVAT was also significantly related with 12-week mortality. HVAT was significantly lower in the mortality group than that in the survival group (9.3±2.0 seconds vs 12.6±3.5 seconds, p=0.002) (Table 1). The odds ratio of HVAT for 12-week mortality was 0.675 (95% confidence interval [CI], 0.478 to 0.953) (Table 2). In other words, when 1 second HVAT was shortened, the mortality risk increased 48.1% (odds ratio, 1.481; 95% CI, 1.050 to 2.090, p=0.025). The AUROC of HVAT for 12-week mortality was 0.787 (p=0.010), it was relatively lower than conventional predictive scoring systems. The sensitivity, specificity, positive predictive value, and negative predictive values, accuracy according to the HVAT cutoff value of <11.0 seconds were 88.9%, 66.7%, 44.4%, 95.2%, and 0.72 respectively. Significant negative correlations were observed between HVAT and Child–Pugh score (R=−0.421, p=0.008), MELD score (R=−0.532, p<0.001), and MDF score (R=−0.547, p<0.001).

In a multivariate analysis based on univariate analysis, HVAT showed significant odds ratio for the prediction of 12-week mortality in each model including Child-Pugh score (model 1), MELD score (model 2) and MDF (model 3) (Table 2).

Table 2. Univariate and Multivariate Analysis for Risk Factors Associated with 12-Week Mortality

| | Univariate analysis | | | Multivariate analysis | | | | | | | | |
| | | | | Model 1 | | | Model 2 | | | Model 3 | | |
	OR	95% CI	p-value	OR	95% CI	p-value	OR	95% CI	p-value	OR	95% CI	p-value
Age	1.006	0.906–1.116	0.918									
Sex, M:F	2.200	0.470–10.302	0.317									
Albumin	0.363	0.083–1.580	0.177									
Total bilirubin	1.119	1.027–1.219	0.10									
Prothrombin time, INR	72.727	4.702–1,124.957	0.002									
Child-Pugh score	2.469	1.172–5.199	0.017	4.046	1.116–14.620	0.033						
MELD score	1.434	1.114–1.847	0.005				2.484	0.994–6.209	0.052			
MDF	1.071	1.023–1.122	0.03							5.090	1.211–122.87	0.0316
HVAT	1.481	1.050–2.090	0.025	1.724	1.132–3.188	0.041	6.905	1.048–68.915	0.046	2.620	1.135–6.047	0.024

Model 1: age, sex, HVAT, Child-Pugh score; Model 2: age, sex, HVAT, MELD; Model 3: age, sex, HVAT, MDF. Multivariate analysis was adjusted by age and sex.
OR, odds ratio; CI, confidence interval; M, male; F, female; INR, international normalized ratio; MELD, Model for End-Stage Liver Disease; MDF, Maddrey's Discriminant Function; HVAT, hepatic vein arrival time.

DISCUSSION

3. The additional predictive value of hepatic vein arrival time for mortality

1) Additional predictive value of HVAT on MDF

Among patients who showed baseline MDF <32, just one death occurred who simultaneously showed HVAT <11.0 seconds. However, no mortality developed in patients who had a HVAT ≥11.0 seconds. Eight mortalities occurred among 21 patients with baseline MDF ≥32, and most (7/8, 87.5%) had a HVAT <11.0 seconds. In contrast, the survival rate was 87.5% when HVAT ≥11.0 seconds, even though the patient initially had a MDF ≥32 (Fig. 3).

2) Additional predictive value of HVAT on MELD

One mortality was combined with a HVAT <11.0 seconds in a patient with baseline MELD <21. However, no mortality occurred in patients who showed HVAT ≥11.0 seconds in the MELD <21 group. Eight deaths occurred in 18 patients with MELD ≥21, and seven of the eight mortalities (87.5%) presented with a HVAT <11.0 seconds. In contrast, 83.3% of patients who had HVAT ≥11.0 seconds survived, even though they initially presented with a MELD ≥21 (Fig. 4).

A number of trials have tried to develop an optimal treatment for AH but only glucocorticoids and pentoxifylline have been accepted as treatment.[17,18] However, their effect does not seem sufficient and their survival benefit has been controversial in the past several decades.[13] Therefore, there is an need for early prediction of a poor prognosis and making a decision for early preparation for LT regardless of the conventional treatment. In general, a MDF value ≥32 has been accepted as SAH and presents a very poor prognosis, with mortality of 20% to 30% within 1 month after development and 30% to 40% within 6 months.[7] Although the MDF has been helpful in predicting the prognosis and decision for treatment, there still has been some discrepancies and difficulties predicting mortality in clinical practice, so several other prognosis scoring systems have been tried.[8-12]

AH is accompanied by hepatic parenchymal necroinflammation, destruction, and neovascularization; these findings are directly related with clinical outcome and the poor prognosis. If AH develops in a patient with underlying cirrhosis, the clinical outcome might be more dismal. Thus, estimating histological severity is essential in the prognostic scoring system; however,
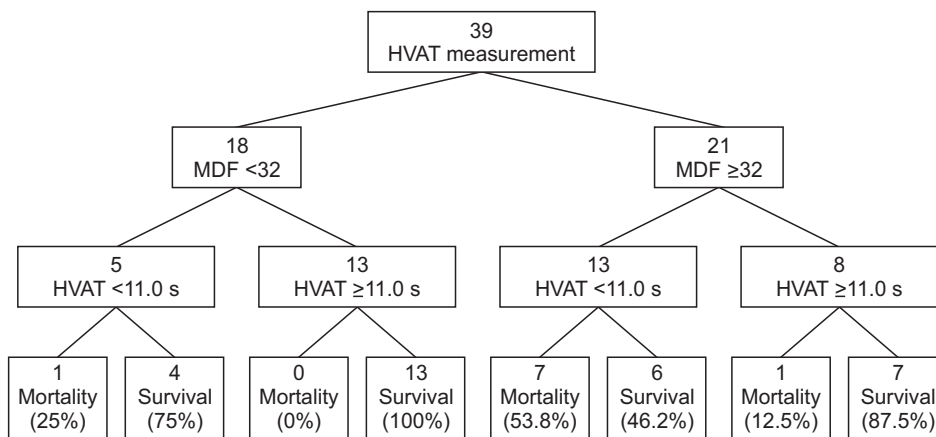

Fig. 3. The additional benefit of using hepatic vein arrival time (HVAT) to make a prognosis based on a Maddrey's Discriminant Function (MDF) score. Among patients with a baseline MDF of <32, only one mortality developed with a simultaneous HVAT of <11.0 seconds. However, when HVAT was ≥11.0 seconds, the survival rate was 87.5%, although the baseline MDF was ≥32.
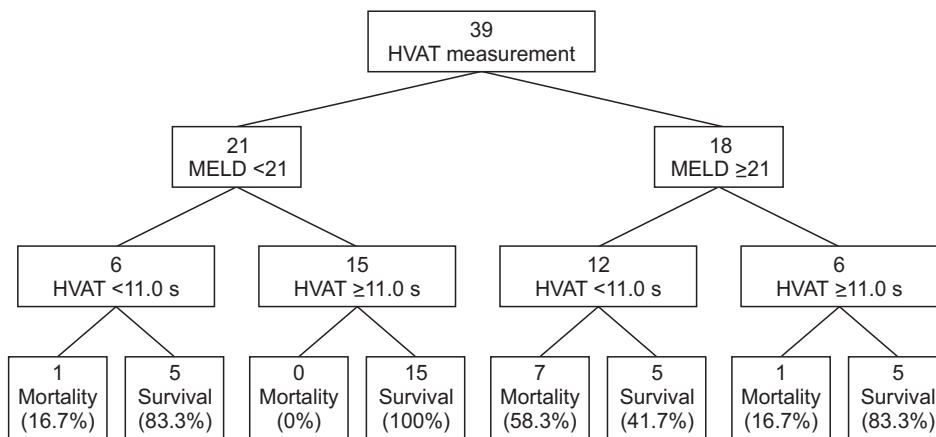

Fig. 4. The additional benefit of using hepatic vein arrival time (HVAT) to make a prognosis based on Model for End-Stage Liver Disease (MELD) score. Only one mortality occurred in patients with a HVAT of <11.0 seconds and a MELD of <21. In contrast, 83.3% of patients with a HVAT of ≥11.0 seconds survived despite initially presenting a MELD of ≥21.

no scoring system includes a histological component. A liver biopsy is not usually recommended for a patient with AH in clinical practice because of its invasiveness and the risk of co-agulopathy. Therefore, developing a noninvasive histological index would improve clinical practice.

The HVAT is the time (in second) taken for the microbubble contrast agent to arrive at the HV after passing through the systemic and intrahepatic circulation. The microbubble ultra-sonography contrast agent normally flows into the liver by two pathways, such as the hepatic artery and the splanchnic circulation-portal vein. After arriving at the liver, the con-trast flows through the intrahepatic sinusoids and reaches the HV. The severity of hepatic fibrosis in chronic liver disease is strongly correlated with early enhancement of the HV and shortening of HVAT,[14,19] and there are sufficient data on the relationship between HVAT and hepatic vein transit time and portal hypertension.[15,16,20] Shortening the HVAT in patients with cirrhosis is secondary to intrahepatic hemodynamic changes, such as arteriovenous shunting, unfavorable neoangiogenesis, and sinusoidal capillarization.[14,19-22] In these conditions, contrast agent bypasses diffusion into a sinusoid and flows directly into the central vein or branch of the HV, so arrival time to the HV is shortened. Shortening of HVAT also reflects deterioration of hepatic function, which is aggravated by impaired liver-tissue oxygenation and hepatic perfusion secondary to intrahepatic histological changes.[23,24] These findings can be more severe when AH is combined with cirrhosis.

In the present study, we evaluated the usefulness of HVAT as a noninvasive index reflecting the intrahepatic histological state in AH. HVAT showed good prognostic value, and a 1 second shortening of HVAT increased the 12-week mortality risk by 48.1%. In particular, the 11.0 seconds HVAT cutoff value is a very unique finding with high sensitivity (88.9%) and negative predictive value (95.2%) for 12-week mortality. This result suggests that HVAT<11.0 seconds can be a useful index to detect high mortality risk patients and to rule out patients who have a relatively low risk of mortality. This is a very important point because classifying patients early who need LT before conventional treatment is critical in clinical practice in the absence of a definitive reliable treatment. Although HVAT did not show superiority in the AUROC compared with traditional prognostic scoring systems to predict 12-week short-term mortality, it was still very useful to combine with the traditional scoring systems. According to the MDF and HVAT combined model, 87.5% of patients who presented with HAVT ≥11.0 seconds survived even though their MDF score was ≥32. In contrast, one of five patients with HVAT <11.0 seconds and MDF <32 died (Fig. 3). Similar findings were observed when using the combined HVAT and MELD model (Fig. 4). These results suggest that HVAT can provide additional prognostic accuracy when combined with a traditional scoring system as a histological severity based index in patients with AH. This combined model can be useful for

early LT before the development of multi-organ failure includ-ing hepatorenal syndrome and acute-on chronic liver failure, especially in a patient with SAH.

This study had some limitations. This was a pilot study, and the small sample size limited clinical adaptation. However, as mentioned above, this study documents the possibility of an ad-ditional effect of HVAT, and a future large-sized well-designed prospective study is warranted to validate the findings. No his-tological findings were documented through liver biopsy. How-ever, sufficient data are available for the correlation between histological and CEUS findings and the ethics issue did not per-mit a highly invasive procedure in patients with AH and a high risk for mortality.

In conclusion, this pilot study prospectively evaluated the clinical usefulness of HVAT. The assessment of intrahepatic histological changes especially vascular changes in AH could be benefit to predict short-term mortality. HVAT has been known to have relation with the acute and chronic histological chang-es, especially vascular changes including neovascularization, and shunting. The present pilot study showed that the combined interpretation of HVAT and conventional scoring systems can be more useful in the prediction of mortality of SAH, especially to detect high risk patients early and exclude low risk patients with SAH.

CONFLICTS OF INTEREST

No potential conflict of interest relevant to this article was reported.

ACKNOWLEDGEMENTS

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