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Daily Monitoring of Serum *Wisteria floribunda* Agglutinin-Positive Mac-2 Binding Protein Is Useful for Predicting Therapeutic Effect of Tolvaptan in Cirrhotic Ascites

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Wisteria floribunda agglutinin (WFA) is a lectin that binds to the sugar chain of Mac-2 binding protein (M2BP), and WFA-positive M2BP (WFA^+-M2BP) has been reported as a useful marker for assessing liver fibrosis in chronic liver disease. Tolvaptan (TLV), a selective vasopressin V2 receptor antagonist, is used for cirrhotic ascites in Japan, but good predictors of treatment efficacy remain to be established. Our aim was to investigate whether WFA^+-M2BP monitoring before and after TLV administration can predict treatment efficacy in patients with cirrhotic ascites. Twenty patients (10 men), with a median age of 72 years, were enrolled. Cirrhosis was caused by hepatitis B virus (n = 3), hepatitis C virus (n = 4), alcohol (n = 8), and others (n = 5). Responders were defined as having a body weight loss of ≥ 1.5 kg/week after TLV administration. Serum WFA^+-M2BP levels were measured at baseline and days 1, 3, and 7 after TLV treatment. Twelve patients (60%) were responders. Baseline WFA^+-M2BP levels were correlated with serum albumin levels ($r = -0.544$, $P = 0.013$). The baseline furosemide dose was lower and platelet count was higher in responders than in non-responders ($P < 0.05$). The ratio of WFA^+-M2BP levels on day 1 after TLV administration to baseline was lower in responders than in non-responders ($P < 0.05$). The decrease in the ratio discriminated responders from non-responders (AUC = 0.844, $P < 0.05$). In conclusion, monitoring serum WFA^+-M2BP is helpful for predicting the efficacy of TLV treatment in patients with cirrhotic ascites.

Keywords: hepatic ascites; liver cirrhosis; tolvaptan; treatment efficacy; *Wisteria floribunda* agglutinin-positive Mac-2 binding protein
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Introduction

Liver cirrhosis is the final manifestation of chronic liver disease. As cirrhosis progresses, patients develop various complications, including esophagogastric varices, hepatic encephalopathy and ascites. Cirrhotic ascites is the most common complication of liver cirrhosis and more than 50% of patients will develop ascites in the 10 years following diagnosis (Ginés et al. 1987). The prognosis of cirrhotic patients with ascites is poor, with a 1-year mortality rate of 15% and a 5-year mortality rate of 44% (Planas et al. 2006). Diuretics are the mainstay of treatment for cirrhotic ascites, and spironolactone, furosemide, and tolvaptan (TLV), a selective vasopressin V2 receptor inhibitor, are commonly used diuretics in Japan.

Spironolactone is currently the first-choice medication for cirrhotic ascites. Furosemide is then used in combination with spironolactone when spironolactone alone is ineffective. TLV can also be used in combination with spironolactone and/or furosemide if they too prove ineffective (Sakaida et al. 2014). The approved indications of TLV use in Japan are fluid retention due to heart failure or liver cirrhosis, and to delay progression of autosomal dominant polycystic kidney. Previous studies have shown that a ther-

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apeutic response is obtained in only 60% of patients treated with TLV (Hiramine et al. 2017; Atsukawa et al. 2020; Sakaida et al. 2020). Several factors that could predict TLV treatment efficacy have been reported. For example, increased blood urea nitrogen levels have been associated with a poor response to TLV, but the cutoff used to assess this varies between studies (Atsukawa et al. 2018; Sakaida et al. 2020). Patients with a higher hepatic venous pressure gradient also had a poor response to TLV (Nakagawa et al. 2016), but measuring hepatic venous pressure is difficult in clinical practice. Therefore, there is a need to identify stable predictive biomarkers that can be easily measured in clinical practice.

Mac-2 binding protein (M2BP) is a secreted glycoprotein, known as a ligand of Mac-2 (Inohara et al. 1996). M2BP contains multi-branching and sialylated *N*-glycans. *Wisteria floribunda* agglutinin (WFA) is a legume lectin that binds *N*-glycan terminals in β-linked *N-acetylgalactosaminide* (Kurokawa et al. 1976; Piller et al. 1990). It has been reported that the change in the sugar chain structure of M2BP recognized by WFA reflects the severity of fibrosis in patients with chronic hepatitis C infection, demonstrating a stepwise increase with an increasing severity of liver fibrosis (Kuno et al. 2013a). Many studies have reported that WFA-positive M2BP (WFA^+-M2BP) is useful for the diagnosis of hepatic fibrosis and prediction of hepatocellular carcinoma (HCC) development in various liver diseases (Yamasaki et al. 2014; Abe et al. 2015; Ishii et al. 2017). Another report investigating changes in WFA^+-M2BP levels in patients with chronic hepatitis C during treatment showed that while WFA^+-M2BP levels were transiently increased and then decreased by interferon-based therapy, they were continuously decreased by interferon-free direct-acting antiviral therapy (Nagata et al. 2016). These results suggest that WFA^+-M2BP levels do not necessarily reflect fibrosis stage and the risk of HCC at the measurement.

Although TLV can adjust body fluid levels in patients with cirrhotic ascites, there are no previous studies investigating temporal changes in WFA^+-M2BP levels during TLV treatment. In the present study, to elucidate the clinical significance of TLV treatment, we monitored serum WFA^+-M2BP levels prior to and after TLV treatment in patients with cirrhotic ascites.

Methods

Patients

This study was an observational study of a prospectively recruited series of 20 liver cirrhotic patients with ascites at Niigata University Hospital between June 2016 and March 2019. Inclusion criteria were as follows: (1) patients aged 20 years and older; (2) patients diagnosed with liver cirrhosis based on imaging studies; and (3) patients with insufficient response to conventional diuretics (loop diuretics, aldosterone antagonists, or both) for at least 7 days with a salt-restricted diet (5-7 g/day), adopted in a Japanese phase 3 study (Sakaida et al. 2014). Exclusion criteria were the presence of the following: (1) esophagogastric varices requiring treatment; (2) overt hepatic encephalopathy; (3) spontaneous bacterial peritonitis; (4) albumin infusion and/or paracentesis of ascites during TLV treatment; and (5) heart failure. The study was in accordance with the 1975 Declaration of Helsinki (2013 revision) and was approved by the institutional review board of the Niigata University Graduate School of Medical and Dental Sciences (No. 2471). Written informed consent was obtained from all study subjects.

Tolvaptan protocol and data collection

All the enrolled patients were admitted, and TLV administration was initiated at a dose of 3.75 mg/day in all patients. Day 0 was defined as the first day of TLV administration. The patients received the same dosage of conventional diuretics on an outpatient basis and maintained on a salt-restricted diet (5-7 g/day), and water intake was not restricted during TLV treatment. If the response was judged not effective during the first 3 days, the dosage was increased to 7.5 mg/day (the maximum dose for cirrhotic ascites) on day 4. In fact, the dosage was increased in three patients, but as a result, all were non-responders. TLV responders were defined as patients with a loss in body weight of 1.5 kg or greater within 7 days of initiating TLV treatment, otherwise they were classified as non-responders (Hiramine et al. 2018). Blood and urine data were obtained at baseline and 1, 3, and 7 days after TLV administration. Serum WFA^+-M2BP levels were measured based on a lectin-antibody sandwich immunoassay using a fully automated analyzer, HISCL-5000 (Sysmex, Hyogo, Japan). WFA^+-M2BP levels were indexed using the following equation: cutoff index (COI) = ([WFA^+-M2BP] of serum sample-[WFA^+-M2BP] of negative control)/([WFA^+-M2BP] of positive control)-[WFA^+-M2BP] of negative control). The positive control was supplied as a calibration solution standardized to yield a COI of 1.0. The range of measurement is 0.1 to 20 COI (Kuno et al. 2013b).

Statistical analysis

All statistics were performed using the SPSS program version 25 (IBM Japan, Tokyo, Japan). Continuous and categorical variables are expressed as medians (range) and numbers, respectively. Changes in values during TLV treatment were analyzed using Friedman's test. Continuous and categorical variables of responders and non-responders were compared using Mann-Whitney *U*-test and Fisher's exact test, respectively. Spearman's rank correlation test was used to determine correlations between WFA^+-M2BP levels before TLV treatment and clinical features. The optimal cut-off value for predicting the response of TLV was determined using a receiver operating characteristic (ROC) curve. All tests were two-sided, and P values < 0.05 were considered statistically significant.

Results

Patient baseline clinical characteristics

Baseline characteristics of the 20 patients are shown in Table 1. There were 10 males and 10 females, with a median age of 72 years (range: 48-85). The etiology of cirrhosis was alcohol in eight patients, hepatitis C virus (HCV) in four patients, hepatitis B virus (HBV), and others in three patient each, and nonalcoholic steatohepatitis in two patients. In 3 HBV-related cirrhosis patients, two received a nucleotide/nucleoside analog therapy and the other did not because of a past HBV infection. In the former, the HBV viral load before TLV treatment was less than 2.1 log copies/mL and undetectable, and in the latter, it was undetectable. In 4 HCV-related cirrhotic patients, the HCV viral loads before TLV treatment were 4.6 log IU/mL in one patient, and 6.2 log IU/mL in three patients. Child-Pugh classification was available for 18 patients because two patients were taking warfarin. Three and 15 patients were classified as grade B and C, respectively. HCC was present in nine patients (45.0 %). Median daily dosages of furosemide and spironolactone before TLV administration were 20 (range: 0-80) mg and 25 (range: 0-100) mg, respectively. Median blood urea nitrogen and serum creatinine levels were 22 (range: 7-61) mg/dL and 1.12 (range: 0.53-2.38) mg/dL, respectively. The median WFA+-M2BP levels were 7.14 (range: 1.32-16.98) COI. Other clinical characteristics of the patients are shown in Table 1.

The baseline WFA+-M2BP levels were positively correlated with aspartate aminotransferase (AST) level ($r = 0.507$, $P = 0.023$), alanine transaminase (ALT) level ($r = 0.508$, $P = 0.022$), total bilirubin level ($r = 0.512$, $P = 0.021$), and prothrombin time-international ratio (PT-INR) ($r = 0.531$, $P = 0.023$), and were negatively correlated with serum albumin level ($r = -0.544$, $P = 0.013$) (Fig. 1).

Table 1. Clinical characteristics before tolvaptan administration.

Variables	n = 20
Age, years	72 (48-85)
Sex, Male/Female	10/10
Etiology of cirrhosis	
Alcohol/HBV/HCV/NASH/Others	8/3/4/2/3
Child-Pugh classification, B/C[a]	3/15
Presence of HCC, yes/no	9/11
Baseline diuretics	
Furosemide, mg/day	20 (0-80)
Spironolactone, mg/day	25 (0-100)
Laboratory data	
AST, U/L	46 (14-100)
ALT, U/L	25 (8-57)
Total bilirubin, mg/dL	1.8 (0.4-8.9)
Serum albumin, g/dL	2.4 (1.7-3.3)
Serum sodium, mEq/L	136 (126-141)
Blood urea nitrogen, mg/dL	22 (7-61)
Serum creatinine, mg/dL	1.12 (0.53-2.38)
Platelet count, $x10^9$/L	109 (34-215)
PT-INR[a]	1.32 (1.18-1.69)
Urine osmolality, mOsm/kg. H_2O	391 (196-677)
WFA+-M2BP, COI	7.14 (1.32-16.98)

Continuous variables are shown as medians (range). Categorical variables are shown as number.
HBV, hepatitis B virus; HCV, hepatitis C virus; NASH, nonalcoholic steatohepatitis; HCC, hepatocellular carcinoma; AST, aspartate aminotransferase; ALT, alanine transaminase; PT-INR, prothrombin time-international ratio; WFA+-M2BP, *Wisteria floribunda* agglutinin-positive Mac-2 binding protein; COI, cut-off index.
[a]Child-Pugh classification status and PT-INR were available for 18 patients.

Effects of TLV treatment on serum WFA+-M2BP levels

Serum WFA+-M2BP levels were measured at baseline and 1, 3, and 7 days after TLV administration. Among the 20 patients enrolled in the present study, there were no significant changes in WFA+-M2BP levels and in WFA+-M2BP ratio from baseline after TLV administration, as judged by the Friedman test (Fig. 2a, b).

We then grouped the patients into TLV responders and non-responders and summarized their baseline clinical characteristics (Table 2): 12 TLV responders (60%) and 8 non-responders (40%). In the baseline WFA+-M2BP levels, there was no significant difference between responders and non-responders. Moreover, serum WFA+-M2BP levels in responders were not significantly different from those in non-responders during TLV treatment (Fig. 3a). Importantly, however, the ratio of WFA+-M2BP levels on day 1 after TLV administration to baseline was significantly lower in responders than that in non-responders ($P = 0.010$, Fig. 3b), but the effect of TLV on the ratio was not detected on day 3 and day 7.

Next, cases of viral (HBV or HCV) and non-viral liver diseases were analyzed separately. Among seven patients with viral (HBV or HCV) liver diseases (see Table 1), six patients with viral liver disease were categorized as the TLV responders (50% of TLV responders). There were no significant differences in serum WFA+-M2BP levels at baseline and after TLV administration (Fig. 4a); namely, WFA+-M2BP levels in patients with viral liver disease were not significantly different from those in patients with non-viral liver disease. In contrast, the day-1-to-baseline ratio of WFA+-M2BP levels in patients with viral liver disease was significantly lower than that in patients with non-viral liver disease ($P = 0.042$, Fig. 4b), but the effect of TLV on the ratio was not detected on day 3 and day 7.

Comparison of other characteristics between TLV responders and non-responders

As shown in Table 2, there was no significant difference in the effect of TLV treatment in patients with or without HCC, which is similar to several reports with a large

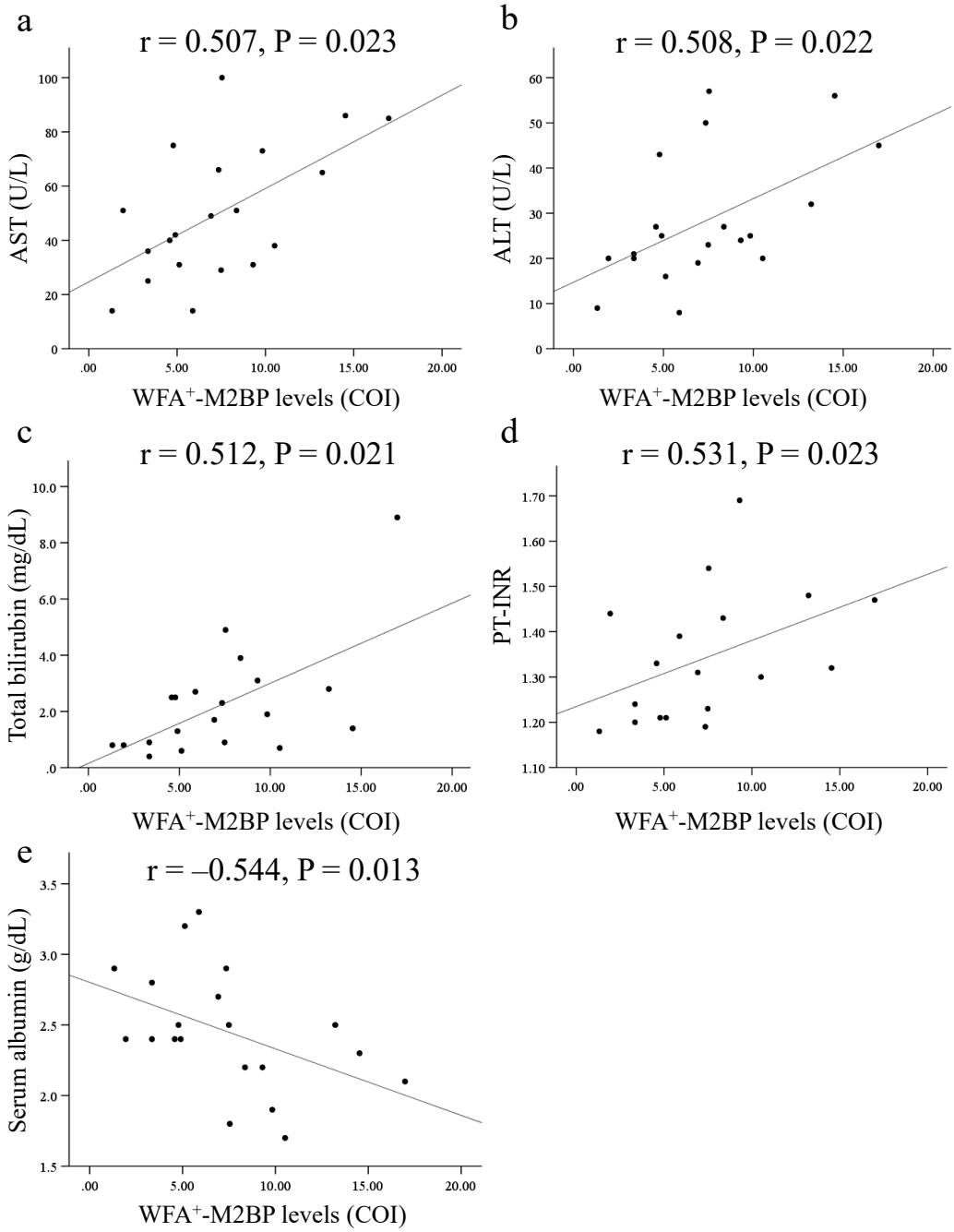

Fig. 1. Correlation between baseline WFA$^+$-M2BP levels and clinical parameters.
Correlation between baseline WFA$^+$-M2BP levels and AST level (a), ALT level (b), T-Bil level (c), PT-INR (d), and serum albumin level (e).
WFA$^+$-M2BP, *Wisteria floribunda* agglutinin-positive Mac-2 binding protein; AST, asparate aminotransferase; ALT, alanine transaminase; T-Bil, total bilirubin; PT-INR, prothrombin time-international ratio; COI, cut-off index.

number of cases (Sakaida et al. 2017, 2020; Hiramine et al. 2017; Atsukawa et al. 2018). The baseline dose of furosemide in responders was significantly lower than that in non-responders (P = 0.010). Platelet count in responders was significantly higher than that in non-responders (P = 0.021).

Prediction of the response of tolvaptan by the day-1-to-baseline ratio of WFA$^+$-M2BP after TLV administration

Finally, we evaluated the performance of the day-1-to-baseline ratio of WFA$^+$-M2BP levels with ROC analysis. ROC analysis revealed that the ratio was predictive of TLV response with area under the ROC curve (AUROC) value 0.844. ROC analysis also identified the rate of 0.985 as the cutoff value to differentiate responders from non-responders (Fig. 5).

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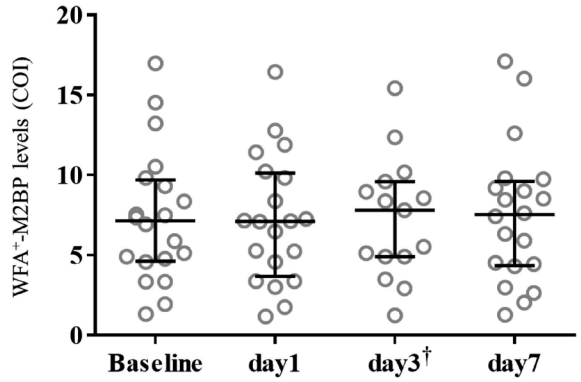
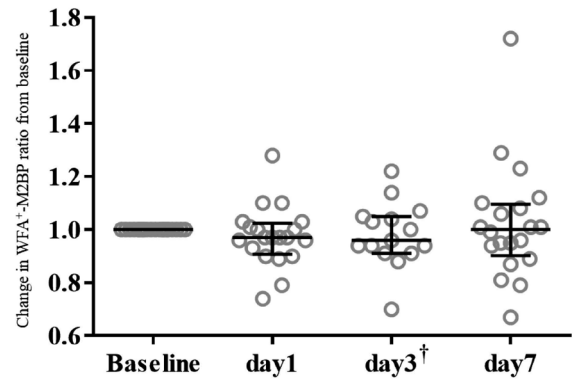


WFA+-M2BP	7.14 (1.32–16.98)	7.11 (1.17–16.44)	7.80 (1.24–15.43)	7.53 (1.27–17.10)

b



Ratio	1	0.97 (0.74–1.28)	0.96 (0.70–1.22)	1.00 (0.67–1.72)

Fig. 2. Changes in WFA+-M2BP levels and WFA+-M2BP ratio in cirrhotic patients treated with tolvaptan.
Median changes in WFA+-M2BP levels (a) and WFA+-M2BP ratio from baseline (b) in 20 patients with cirrhotic ascites treated with tolvaptan. The median with interquartile ranges are shown in bars.
WFA+-M2BP, *Wisteria floribunda* agglutinin-positive Mac-2 binding protein; COI, cut-off index.
†n = 15.

Discussion

In the present study, we investigated the usefulness of WFA+-M2BP monitoring before and after TLV administration in predicting its treatment efficacy in patients with cirrhotic ascites. The correlation among the following factors was first analyzed: baseline WFA+-M2BP levels and total bilirubin, PT-INR, and serum albumin, which are factors of the Child-Pugh score, a traditional method for estimating hepatic functional reserve (Pugh et al. 1973). As expected, there were positive correlations among baseline WFA+-M2BP levels, total bilirubin level, and PT-INR, and a negative correlation between WFA+-M2BP levels and serum albumin level. Furthermore, the WFA+-M2BP levels were positively correlated with levels of AST and ALT, which are markers of liver inflammation. These results suggest that WFA+-M2BP levels are increased in patients with hepatic functional reserve deterioration or severe inflammation; histological analysis was not performed in this study (Haga et al. 2016; Nishikawa et al. 2016; Ishii et al. 2017).

Among patients with TLV treatment, furosemide administration doses were lower in TLV responders than in non-responders. It has been reported that the glomerular filtration rate is decreased with a furosemide treatment dose of 40 mg/day in patients with cirrhotic ascites, and a further increase in dosage may induce an underfilling state by reducing the circulatory volume, resulting in severe refractory ascites and/or acute renal failure (Bernardi et al. 1985; Tsien and Wong 2013). Therefore, TLV is recommended for ascites treatment in Japan before renal dysfunction

Table 2. Comparison of clinical characteristics in responders and non-responders to tolvaptan.

Variables	Responders (n = 12)	Non-responders (n = 8)	P value
Age, years	75 (48-85)	68 (54-84)	0.175
Sex, Male/Female	7/5	4/4	> 0.999
Etiology of cirrhosis			
Alcohol/HBV/HCV/NASH/Others	4/3/3/0/2	4/0/1/2/1	0.294
Child-Pugh classification, B/C[a]	1/9	2/6	0.559
Presence of HCC, yes/no	6/6	3/5	0.670
Baseline diuretics			
Furosemide, mg/day	20 (0[b]-80)	40 (20-40)	0.010
Spironolactone, mg/day	50 (0[b]-100)	25 (0[b]-50)	0.190
Laboratory data			
AST, U/L	47 (25-100)	40 (14-75)	0.231
ALT, U/L	25 (20-57)	22 (8-43)	0.132
Total bilirubin, mg/dL	1.4 (0.4-8.9)	2.6 (0.6-3.9)	0.354
Serum albumin, g/dL	2.4 (1.7-2.9)	2.6 (2.2-3.3)	0.057
Serum sodium, mEq/L	136 (126-141)	137 (128-140)	0.726
Blood urea nitrogen, mg/dL	22 (7-52)	21 (13-61)	0.908
Serum creatinine, mg/dL	1.23 (0.53-1.88)	0.95 (0.57-2.38)	0.847
Platelet count, $\times 10^9$/L	124 (34-215)	72 (35-120)	0.021
PT-INR[a]	1.31 (1.19-1.54)	1.35 (1.18-1.69)	0.859
U-OSM, mOsm/kg. H_2O	387 (303-677)	391 (196-584)	0.671
Decrease in U-OSM at 4h after TLV administration, %	40.6 (1.2-59.1)	19.2 (−0.4-69.3)	0.105
WFA^+-M2BP, COI	7.42 (1.94-16.98)	6.40 (1.32-13.22)	0.817

Continuous variables are shown as medians (range). Categorical variables are shown as number.
HCC, hepatocellular carcinoma; AST, aspartate aminotransferase; ALT, alanine transaminase; PT-INR, prothrombin time-international ratio; U-OSM, Urine osmolality; TLV, tolvaptan.
[a]Child-Pugh classification status and PT-INR were available for 18 patients.
[b]Tolvaptan is used in combination with spironolactone and/or furosemide.

develops due to excess treatment with furosemide, which is different from the recommendation in the United States and the European Union. In previous studies, platelet counts in TLV responders were not significantly different from those in non-responders (Uojima et al. 2017; Atsukawa et al. 2018, 2020). However, in the present study, platelet counts were found to be higher in responders than in non-responders. This discrepancy in results may be attributed to the smaller sample size of the present study.

It is known that the absolute values of WFA^+-M2BP level of advanced hepatic fibrosis vary depending on the etiology of liver cirrhosis. For example, the median WFA^+-M2BP level of HCV cirrhosis was higher than that of cirrhosis of HBV, primary biliary cholangitis or nonalcoholic steatohepatitis (Yamasaki et al. 2014; Abe et al. 2015; Ishii et al. 2017; Shirabe et al. 2018). In the present cohort, the etiology of cirrhosis was heterogeneous resulting in various WFA^+-M2BP levels. Therefore, we analyzed the changes in WFA^+-M2BP levels from baseline after TLV treatment in addition to the absolute values of WFA^+-M2BP level. In the present study, we found that the reduction rate of WFA^+-M2BP level from the baseline at day 1 after TLV treatment was a potential predictor of the therapeutic effect of TLV, suggesting that assessing changes in WFA^+-M2BP levels, not absolute values, are important for treatment effectiveness. The precise mechanisms whereby serum WFA^+-M2BP levels change immediately after TLV administration remain speculative. Previous studies have shown that the hepatic venous pressure gradient and renin-angiotensin-aldosterone system were not significantly changed between pre- and post-TLV treatment (Costello-Boerrigter et al. 2006; Nakagawa et al. 2016). Another study showed that WFA^+-M2BP was secreted by hepatic stellate cells (Bekki et al. 2017). Hepatic stellate cells have cross talk with various cells (e.g., Kupffer cells and sinusoidal endothelial cells) within a hepatic microenvironment (McConnell and Iwakiri 2018). It is speculated that short-term changes in WFA^+-M2BP levels reflect changes in body fluid at the cellular level of the hepatic microenvironment in TLV responders; however, it is unknown if the changes are a direct or indirect effect on the stellate cells. Further detailed analyses are necessary. We also found that the ratio of WFA^+-M2BP levels from baseline to day 1 after TLV administration in patients with viral liver disease was significantly lower than that in patients with non-viral liver disease. In this cohort, six out of seven patients with viral

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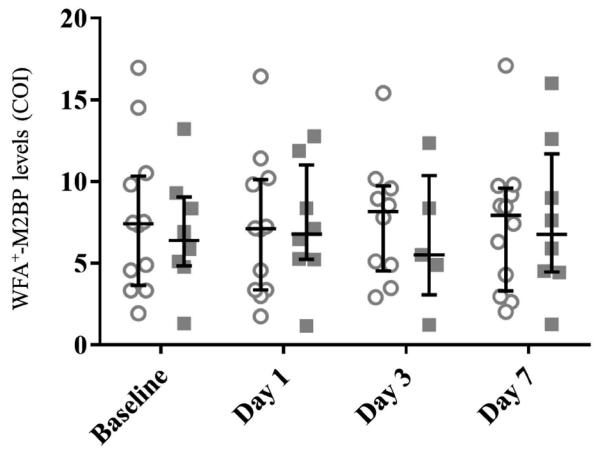

Responder (n =12)	7.42 (1.94–16.98)	7.13 (1.75–16.44)	8.18 (2.93–15.43)†	7.94 (2.05–17.10)
Non-responder (n = 8)	6.40 (1.32–13.22)	6.80 (1.17–12.77)	5.52 (1.24–12.36)‡	6.77 (1.27–16.03)
P value	0.817	0.817	0.624	> 0.999

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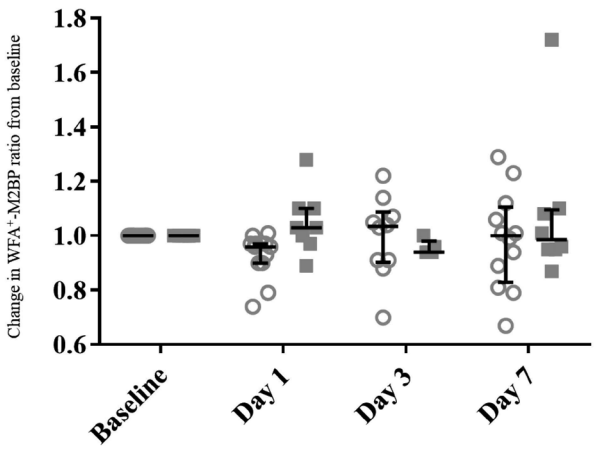

Responder (n = 12)	1	0.96 (0.74–1.01)	1.04 (0.70–1.22)†	1.00 (0.67–1.29)
Non-responder (n = 8)	1	1.03 (0.89–1.28)	0.94 (0.94–1.00)‡	0.99 (0.87–1.72)
P value	–	0.010	0.538	0.588

Fig. 3. Comparison of changes in WFA+-M2BP levels and WFA+-M2BP ratio between TLV responders and non-responders. Median changes in WFA+-M2BP levels (a) and WFA+-M2BP ratio from baseline (b) in 20 patients with cirrhotic ascites after tolvaptan treatment. The open circles and closed squares indicate responders and non-responders, respectively. The median with interquartile ranges are shown in bars. The Friedman test revealed no significant changes within each group over time.
WFA+-M2BP, *Wisteria floribunda* agglutinin-positive Mac-2 binding protein; COI, cut-off index.
†n = 10; ‡n = 5.

liver disease were TLV responders, which were considered to be influenced. In fact, in three studies with a large number of cases, there was no significant difference in the treatment effect in patients with viral and non-viral liver diseases (Hiramine et al. 2017; Atsukawa et al. 2018; Sakaida et al. 2020).

There are several limitations to the present study. First, the present study is a small-sized single-center study in which only 20 patients were included. Blood urea nitrogen was not found as a predictive factor of treatment effect, as reported previously (Sakaida et al. 2020), which may have been due to the small sample size. Second, in the present cohort study, many enrolled patients displayed relatively preserved renal function. This may reflect our strict adherence to the recommended treatment in which concomitant TLV treatment was initiated before deterioration of

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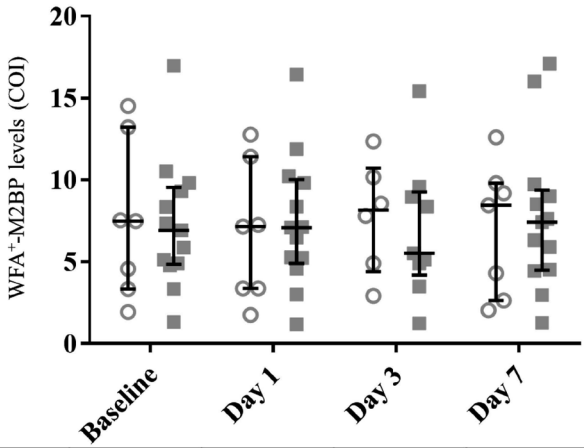

Viral (n = 7)	7.49 (1.94–14.53)	7.15 (1.75–12.77)	8.18 (2.93–12.36)†	8.46 (2.05–12.60)
Non-viral (n = 13)	6.92 (1.32–16.98)	7.10 (1.17–16.44)	5.52 (1.24–15.43)‡	7.42 (1.27–17.10)
P value	0.937	0.905	0.724	0.905

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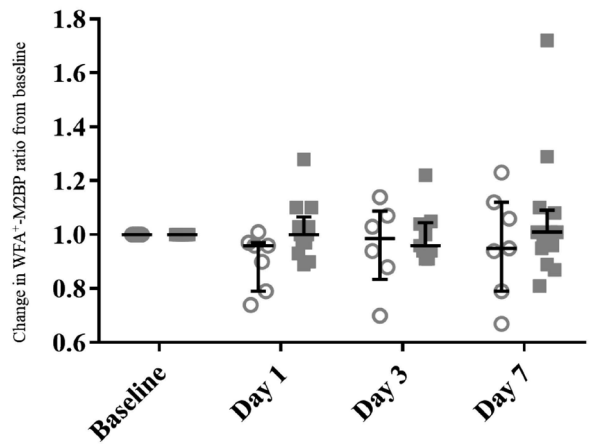

Viral (n =7)	1	0.96 (0.74–1.01)	0.99 (0.70–1.14)†	0.95 (0.67–1.23)
Non-viral (n = 13)	1	1.00 (0.89–1.28)	0.96 (0.91–1.22)‡	1.01 (0.81–1.72)
P value	–	0.042	0.813	0.525

Fig. 4. Comparison of changes in WFA$^+$-M2BP levels and WFA$^+$-M2BP ratio in cirrhotic patients between viral and non-viral liver diseases.
Median changes in WFA$^+$-M2BP levels (a) and WFA$^+$-M2BP ratio from baseline (b) in 20 patients with cirrhotic ascites after tolvaptan treatment. The patients were divided into groups with viral (n = 7) and non-viral (n = 13) liver disease. The open circles and closed squares indicate viral and non-viral liver disease, respectively. The median with interquartile ranges are shown in bars.
WFA$^+$-M2BP, *Wisteria floribunda* agglutinin-positive Mac-2 binding protein; COI, cut-off index.
†n = 6; ‡n = 9.

renal function induced by the excess doses of loop diuretics. Third, TLV is inconvenient to use as it must be taken at least once to judge its therapeutic effect. A simple marker that can predict therapeutic effects before administration is a future concern. Fourth, the TLV dose was not consistent among the patients. Three patients whose TLV treatment response was insufficient received an increased TLV dose of 7.5 mg/day on treatment day 4. However, all were non-responders. The remaining 17 patients received TLV at a dose of 3.75 mg/day until assessment of the treatment effect. Therefore, careful interpretation of the results of the present study is necessary. Further studies with more cases are necessary to evaluate the results of the present study.

In conclusion, the reduction rate of WFA$^+$-M2BP levels from the baseline to day 1 after TLV treatment could be predictive of the TLV treatment effect. Though there are still several limitations, it is conceivable that the management of cirrhotic ascites can be performed with the appro-

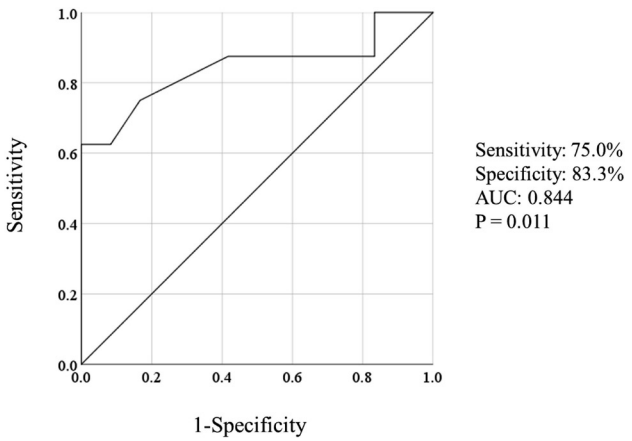

Fig. 5. Receiver operating characteristic curves for predicting response to tolvaptan in 20 patients with cirrhotic ascites. The curve shows the ratio of WFA+-M2BP levels on day 1 after tolvaptan treatment to baseline.
AUC, area under the curve.

priate use of TLV, and its efficacy may be monitored with serum WFA+-M2BP levels.

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Author Contributions

M.T. and S.T. conceived the study design. M.T., A.S., Y.A., T.S., H.K., T.Y., K.K., and A.T. collected samples. M.T. analyzed the data and wrote the manuscript. S.T. reviewed the manuscript.

Conflict of Interest

The authors have no conflict of interest concerning the present study. On the other hand, S. Terai received lecture fees from Otsuka Pharmaceutical Co., Ltd. and research funding from Sysmex.

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