Increased serum C-reactive protein and decreased urinary aquaporin 2 levels are predictive of the efficacy of tolvaptan in patients with liver cirrhosis

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Increased serum C-reactive protein and decreased urinary aquaporin 2 levels are predictive of the efficacy of tolvaptan in patients with liver cirrhosis

Short title: Predictive factors of tolvaptan efficacy for LC

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4 tables (One is a supplemental table)
Abstract

**Aim:** Water retention, hepatic ascites, and peripheral edema are significant problems in patients with liver cirrhosis (LC). Although furosemide and spironolactone are commonly used as treatment, these are often insufficient to treat hyponatremia and renal insufficiency in patients with LC. Tolvaptan (TVP) could provide an effective treatment alternative. However, predictive factors of a therapeutic response to TVP are unclear. Our aim was to examine clinical predictors of the response to TVP in patients with LC and water retention.

**Methods:** Fifty-two patients were treated with TVP, with therapeutic effects judged by a decrease in body weight (≥2 kg) and increase in urinary volume (≥500 ml) within 7 days. Blood biochemical tests were performed at baseline and post-administration, including serum soluble CD14 (sCD14) and urinary aquaporin 2 (AQP2) levels. Clinical and laboratory predictive factors of a TVP response were evaluated by univariate and multivariate analyses.

**Results:** The overall response to TVP was 55.8%. On univariate analyses, serum C-reactive protein (CRP) level, the neutrophil-to-lymphocyte ratio, urinary BUN, and urinary AQP2 were predictors of the TVP response, with only serum CRP retained on multivariate analysis. A higher serum sCD14 level was strongly associated with a non-response to TVP. A decrease in urinary AQP2 to undetectable level was associated with a response.

**Conclusion:** TVP provides a rapid and strong effect to improve water retention in patients with LC. Baseline serum sCD14 and CRP levels are useful predictors of a response to TVP, with a decrease in urinary AQP2 during treatment indicating an early response.
Key words: Tolvaptan; water retention; CRP; soluble CD14; urinary aquaporin 2
Introduction

Patients with decompensated liver cirrhosis (LC) develop numerous complications, such as gastroesophageal varices, hepatic encephalopathy, and water retention. Such complications substantially compromise the quality of life of these patients. In particular, water retention involving peripheral edema, pleural fluid, and ascites are frequently observed in patients with decompensated LC and are sometimes difficult to control. Although not fully understood, there are several hypotheses regarding possible mechanisms of water retention in patients with LC, such as the underfilling hypothesis, the overfilling hypothesis and the vaso-dilative hypothesis. For all of these, arginine vasopressin (AVP) plays a key role in water retention. AVP has two principal functions: body water retention and vasoconstriction. There are two types of receptors for AVP: AVP-V1 and AVP-V2. The V1 receptor is expressed in the vascular endothelium and mediates vasoconstriction. The V2 receptor is expressed in the renal collecting ducts and facilitates free water reabsorption.

Furosemide and spironolactone have been used to control water retention in patients with long-term decompensated LC. These diuretics help to excrete sodium but sometimes induce renal function failure. Acute renal function results in a poor prognosis for patients with decompensated LC. Furthermore, hyponatremia is a poor prognostic factor in this clinical population.

Tolvaptan (TVP), an AVP-V2 selective antagonist, binds to AVP-V2 receptors and inhibits reabsorption of water via aquaporin 2 (AQP2). This mechanism occurs by inhibiting the intracellular movement of AQP2 to the cytoplasmic membrane on the luminal surface of renal collecting ducts. Because
TVP inhibits reabsorption of free water via AQP2 but not via the sodium channel, hyponatremia, which is a major adverse event of sodium excretion caused by furosemide and spironolactone, does not develop. As approximately 3% of AQP2 is excreted in urine as an exosome, changes in the AQP2 level in urine reflect the function of AVP.

Because of the novel mechanism of action and high efficiency, TVP has been approved for clinical use in Japan and is widely prescribed to treat water retention in patients with LC, improving the prognosis of patients with LC during a stage when renal function is maintained. However, TVP is not effective for all patients. In patients with heart failure, several predictors of the response to TVP have been reported, such as urinary osmolality and urinary AQP2 levels. However, little is known about the predictors of the response to TVP for patients with LC.

Serum soluble CD14 (sCD14) is cleaved and released from membrane-bound CD14 on peripheral blood mononuclear cells by inflammatory responses of lipopolysaccharides, with serum levels of sCD14 increasing as chronic liver disease progress. Furthermore, higher sCD14 is associated with bacterial translocation (BT). Moreover, BT in patients with decompensated LC promotes portal hypertension and induces variceal bleeding, hepatorenal syndrome and hepatic encephalopathy.

Our aim in this study was to evaluate patients with decompensated LC treated with TVP to determine clinical predictors of a response to TVP in patients with LC and water retention.
Methods

Patients

Fifty-two consecutive patients with decompensated LC and water retention were treated with TVP, from October 2013 to December 2016, at Hokkaido University Hospital. Patient inclusion criteria were LC and refractory water retention (ascites, pleural effusion, peripheral edema) which was not controlled by treatment with other diuretics (furosemide, spironolactone). Exclusion criteria were malignant ascites, dominant hepatic encephalopathy, uncontrollable fever, heart failure, second administration of TVP, active gastroesophageal bleeding, and presence of a dominant infectious disease.

TVP was administered once daily. The starting dose was 3.75 mg for 44 patients (84.6%) and 7.5 mg for 8 patients (15.4%; Table 1). If the response to TVP was poor, then the dose was increased after 3-4 days. Body weight was measured in the morning for all patients and water intake was not restricted.

Blood chemistry data were obtained at baseline, and at 6 h, 24 h and 7 days after TVP initiation. Urine chemistry was obtained at baseline, and at 6 h and 24 h after TVP initiation. All adverse events were evaluated using the common terminology criteria for adverse events (CTCAE) version 4.0.

Measurements of sCD14

sCD14 was measured using the sandwich ELISA method, with the Human sCD14 Quantikine ELISA Kit (R&D Systems), from conserved serum samples obtained at baseline. Three measurements were performed three times and the average value was used. sCD14 levels were obtained in 37 patients whose
conserved serum from baseline was available.

Measurement of AQP2

Urinary AQP2 was measured, at baseline and at 6 h post-TVP administration, using a sandwich ELISA method (LSI Medience Corporation, Japan), with a cutoff value of 0.61 pg/ml.

Ethical considerations

The study protocol was approved by the institutional ethics committee of Hokkaido University and conformed to the ethical guidelines of the Declaration of Helsinki. The study was registered with the UMIN Clinical Trials Registry as UMIN 000021300.

Statistical analysis

Statistical analyses were performed using EZR.\textsuperscript{18} A Mann-Whitney U test was used for analysis of continuous variables. Fisher’s exact test used for univariate analysis of ordered variables. Logistic regression and multivariate analyses were used for ordered variables. Spearman’s rank correlation coefficient was used to evaluate the correlation between variables.
Results

Patient characteristics

Fifty-two patients with LC were treated with TVP between October 2013 and December 2016, and observed for more than 1 week after TVP administration. Relevant characteristics of these patients are described in Table 1, and summarized as follows. The median age was 65 years, with 69.2% of the study group being men. Regarding background liver diseases, 9 patients had hepatitis C virus and 18 hepatitis B virus, with the remaining 25 having non-B non-C liver disease. Twenty-three patients had advanced hepatocellular carcinoma (HCC), based on the Milan criteria. The median Child-Pugh score was 10 points, and the Model for End-Stage Liver Disease (MELD) score was 10.85 points. The starting dose of TVP was 3.75 mg for 44 patients and 7.5 mg for 8 patients. Furosemide (20 mg; median dose) and spironolactone (50 mg) were used together as diuretics. Ascites or pleural fluid were examined in 27 patients in whom a puncture could be safely performed. None of these patients presented symptoms of abdominal cavity infection, and the culture of ascites or pleural fluid was negative, with a white blood cell count (WBC) of < 500 per μl. Therefore, none of the patients had symptomatic and culture-positive SBP. The median administration period of TVP was 36 days.

Efficiency of TVP for liver cirrhosis

A therapeutic response to TVP was defined by a decrease in body weight >2 kg and an increase in urinary volume of >500 ml relative to baseline, before the treatment, within 1 week of TVP administration, without the need for drainage of
ascites or plural effusion. This definition was used because the median decrease in body weight at 1 week was approximately 2 kg during a phase III clinical trial in Japan.9

As shown in Figure 1A, the response rate to TVP was 55.8% (29/52 patients), with an average decrease in body weight of approximately 2 kg achieved. The urinary volume peaked at 1 day post-TVP administration, with an average increase in urinary volume of approximately 1200 ml (as shown in Figure 1B). Seven patients required drainage of ascites or plural effusion, and 8 patients required an increased TVP dose within 1 week. There was no change in serum sodium levels in almost all patients (Figure 1C).

*Predictive factors for a response to TVP*

To clarify the characteristic of TVP responders, we compared the background characteristics, as well as blood and urine data obtained before TVP administration, between responders and non-responders. As described in Table 2, the CRP, neutrophil-to-lymphocyte ratio, urinary blood urea nitrogen (BUN), urinary AQP2, and the urinary AQP2/Cr ratio were significantly different between responders and non-responders. Receiver operating curve (ROC) analysis identified a CRP level of 0.9 mg/dl as the best cutoff value to differentiate responders from non-responders (Figure 2). A CRP level ≥0.9 g/dl was confirmed to be significantly different between responders and non-responders on multivariate analysis (Table 3). Therefore, the CRP level before treatment is a good predictor of a therapeutic response to TVP, with a high CRP level being an important factor of a poor response to TVP.
**sCD14 level and CRP in non-responders**

To determine the cause of higher CRP levels in non-responders, we focused on latent bacterial translocation (BT). A previous study has reported increased levels of BT biomarkers among patients in end-stage liver disease.\(^\text{17}\) As sCD14 was recently proposed as a good surrogate marker of BT,\(^\text{17}\) we examined sCD14 levels in 37 patients for whom conserved serum samples from baseline were available. As shown in Figure 3 (A and B), the CRP level was correlated to the sCD14 level in all patients, including those without a diagnosis of advanced HCC, as per the Milan criteria. Moreover, sCD14 levels were significantly lower in TVP responders than non-responders (Figure 4). Therefore, patients with latent BT may have higher CRP and resist TVP therapy during LC.

**Change in urinary AQP2 after TVP treatment**

Because urinary AQP2 and osmolality were previously reported to be predictive factors of TVP response among patients with heart failure, we studied whether these factors were also useful for predicting a response to TVP in patients with LC. As described in Tables 2 and 3, urinary osmolality and AQP2 level at baseline were not good predictors of a response to TVP. When TVP works as an antagonist of AVP-V2 receptors in the renal collecting duct, the excretion of AQP2 is expected to decrease. Therefore, we measured changes in AQP2 and the AQP2/Cr ratio after TVP administration (Figure 5A and B). Urinary AQP2 and the AQP2/Cr ratio at 6 h post-TVP administration were significantly lower in TVP responders than in non-responders, decreasing to almost undetectable in almost all...
responders. Based on these results, it is possible that urinary AQP2 at 6 h post-TVP administration predicts an early response to TVP.

Adverse events

Six patients had adverse events: 1 developed anorexia (grade 1), 2 orthostatic hypotension (grade 1), 2 hepatic coma (grade 2), and 1 renal dysfunction (grade 3). All patients recovered and no resulting increases in physical disorders observed.
Discussion

TVP is a selective vasopressin-V2 receptor antagonist that works in the collecting tube of the kidney and inhibits reabsorption of water. This drug is available for heart failure in Japan and for syndromes of inappropriate antidiuretic hormone secretion in the United States. TVP has good effectiveness and is somewhat protective against heart failure.14,15 TVP has been available for treating water retention resulting from LC since September 2013 in Japan. There is no specific definition of TVP efficacy, so several parameters, such as body weight and urinary volume, are used. Because the average decrease in body weight was approximately 2 kg/week during a phase III study in Japan,9 we defined responders as those with a decrease in body weight ≥2 kg and an increase in urinary volume ≥500 ml within 1 week. The rate of efficacy was approximately 56%, which is consistent with previous reports.

Several factors (decreases in urinary osmolality, serum BUN, serum creatinine, serum BUN, urinary BUN excretion, urinary Na excretion and urinary Na/K ratio) were previously reported as predictive factors of TVP response among patients with LC.22-29 In our study, CRP, neutrophil-to-lymphocyte ratio, urinary BUN and urinary AQP-2 were identified as predictive factors on univariate analysis, based on the data of all 52 patients in our study group. A CRP level <0.9 mg/dl was the best predictive factor on multivariate analysis. If higher CRP affects the response to TVP, then it is necessary to consider the factors that cause an increase in CRP levels. We considered that higher CRP might be derived from latent BT due to inflammation of the liver or abdominal cavity. As CRP is closely correlated to sCD14 (Figure 2), we evaluated sCD14 data by multivariate analysis
for the 37 patients in whom conserved baseline serum was available. We identified a sCD14 level ≥1665 pg/ml to be a significant predictive marker of TVP efficacy (Supplemental Figure 1 and Supplemental Table 1). Based on our results, we propose that the sCD14 level, which was correlated to the CRP level, might be the most meaningful factor of TVP response. However, it is important to note that our study sample included a small number of patients and, therefore, a larger cohort study is needed to confirm our findings.

CD14 is an important receptor of lipopolysaccharide (LPS).\textsuperscript{30} It exists in membrane-bound CD14 (mCD14), which is mainly expressed on the surface of monocytes, macrophages and immune cells. In the liver, Kupffer and sinusoidal endothelial cells express mCD14, which works as a co-receptor of TLR4, recognizing LPSs and inducing interferon and inflammatory cytokines. Part of this CD14 exists as a soluble form (sCD14) in blood.\textsuperscript{31, 32} As the sCD14 level increases with progression of liver diseases,\textsuperscript{16} it is assumed to be a good marker of BT.\textsuperscript{17} Furthermore, serum CRP may be a marker of BT in some instances.\textsuperscript{33} In our study, serum levels of CRP and sCD14 were positively correlated, and patients with higher sCD14 had a poor response to TVP treatment. In contrast, SBP is often complicated with BT from the intestinal tract to the mesenteric lymph nodes in patients with decompensated LC,\textsuperscript{34, 35} with the SBP condition being associated with a poor response to diuretics. As CRP was previously identified as a diagnostic marker of SBP, we speculated that BT causes non-symptomatic and culture-negative SBP, which may result in a poor response to TVP. In our study, the culture of ascites or pleural fluid was negative in all patients examined. However, we could not strictly exclude culture-negative SBP because the
neutrophil count of ascites was not available. Although examination of ascites revealed that sCD14 and CRP levels did not correlate with WBC count of ascites in 27 patients who were evaluated (Supplemental Figure 2), we speculated that levels of CRP or sCD14 might correlate to the neutrophil count of ascites. However, only half of the patients underwent ascites or pleural fluid examination and the neutrophil count was not available for these patients. Therefore, further study of a larger cohort including ascites or pleural fluid examination and neutrophil count is needed to confirm our results.

In our study, the decrease in urinary AQP2 was also found to be predictive of the TVP response. AQP2 functions as a water channel, depending on the number of AVP receptors at the collecting duct of the renal interstitium. When AVP attaches to receptors, the water channel moves to the luminal surface of the collecting duct and resorbs water.12 Approximately 3% of cellular AQP2 is excreted in urine.11 The amount of AQP2 in the water channel can be estimated by measuring the amount of urinary AQP2. Urinary AQP2 concentration and urine osmolality have previously been reported as predictive factors of a response to TVP in patients with heart failure patients.14, 15 Imamura et al.14 reported the AQP2/AVP ratio is also predictive marker of TVP response in heart failure patients. In our study, baseline AVP and urinary AQP2 levels tended to be lower in non-responders than in responders, although the AQP2/AVP ratio was not different between the two groups and was not predictive of a TVP response. It has previously been reported that both AVP and AQP2 levels increase with progression of LC, and that the level of AQP2 was also correlated to the degree of ascites.36-38 It is therefore possible that a large decrease in AQP2 with TVP administration
might be difficult to achieve in patients with high AVP and AQP-2 levels at baseline, with an overall poor response to TVP.

In our study, urinary AQP2 and urine osmolality had a strong positive correlation (data not shown). Although these factors are predictive of the TVP response, both these factors were not retained as independent predictors of TVP response in patients with LC on multivariate analysis. However, a decrease in AQP2 level or the AQP2/Cr ratio to an undetectable level at 6 h post-TVP administration was an early predictor of a good response to TVP. This result was consistent with a previous report of greater body weight reduction in patients with LC who were AQP2 responders, where AQP2 response was defined as a AQP2/Cr ratio of “0” at 4 h post-TVP administration. In our study, we also identified the greatest reduction in body weight among AQP2 responders (Supplemental Figure 3). Because most patients who responded to TVP showed a decrease in AQP2 to an undetectable level at 6 h post-TVP administration, we propose that AQP2 is a good index for determining whether TVP administration should be continued during the early stages of therapy.

The limitations of our study need to be acknowledged. Foremost, this was a retrospective observational study involving a single hospital and a small number of patients. The examination of ascites/pleural fluid was evaluated in only 27 patients, and sCD14 was evaluated only in 37 patients for whom the neutrophil count was not available. A prospective study including a large number of patients and ascites examination from many institutions is necessary in the future.

In conclusion, our study showed that serum CRP correlated with sCD14, derived from latent BT, and was a good predictor of the response to TVP in
patients with LC. Decreased excretion of urinary AQP2 is also a good predictor of the response to TVP. Therefore, we may be able to predict the effects of TVP by using these factors together.

Acknowledgments

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Figure Legends

Figure 1. Changes in body weight, urinary volume, and serum sodium after administration of tolvaptan.

(A) Changes in body weight after starting tolvaptan treatment are shown on the Y-axis. (B) Changes in urinary volume after starting tolvaptan treatment are shown on the Y-axis. (C) Changes in serum sodium after starting tolvaptan treatment are shown on the Y-axis. In each graph, plots represent mean ± SD.

Figure 2. ROC curve analysis of CRP and prediction of weight loss.

The ROC curve, showing the baseline CRP predictive of a weight reduction ≥2 kg within 7 days after tolvaptan treatment.

Figure 3. Correlation between serum CRP and sCD14.

(A) Correlation between serum CRP and sCD14 for 37 patients for whom sCD14 measurements were possible. (B) Correlation between CRP and sCD14 for 23 patients who did not have advanced HCC, as per the Milan criteria. The box charts for the X-axis and Y-axis indicate the median and 25 and 75 percentiles as boxes, the first quartiles − 1.5 × IQR (interquartile range), and the third quartiles + 1.5 × IQR as lines outside the boxes.

Figure 4. sCD14 level for responders and non-responders to tolvaptan.

sCD14 values before tolvaptan treatment for responders and non-responders; the bold solid line indicates the median.
Figure 5. Changes in urinary AQP2 and urinary AQP2/Cr after tolvaptan administration in responders and non-responders to tolvaptan.

Urinary AQP2 and the urinary AQP2/Cr ratio at 6 h post-TVP administration are shown on the Y-axis. (A) Urinary AQP2 of responders and non-responders. (B) The urinary AQP2/Cr ratio of responders and non-responders. The box charts for the Y-axis indicate the median as bold lines in the boxes, 25th and 75th percentiles as boxes, 10th and 90th percentiles as lines for each edge.

Supplemental Figure 1. ROC curve analysis of sCD14 and prediction of weight loss.

The ROC curve, showing the baseline sCD14 predictive of a weight reduction ≥2 kg within 7 days post-TVP administration in 37 patients.

Supplemental Figure 2. Scatter diagram of WBC count of ascites/pleural fluid and serum CRP / sCD14.

(A) Scatter diagram of WBC count of ascites or pleural fluids and CRP in 27 patients who were able to undergo ascites measurements. (B) Scatter diagram of WBC count of ascites or pleural fluids and sCD14 in 14 patients who were able to undergo ascites examination and sCD14 measurements. The box charts for the X-axis and Y-axis indicate the median and 25 and 75 percentiles as boxes, the first quartiles − 1.5 × IQR (interquartile range), and the third quartiles + 1.5 × IQR as lines outside the boxes.
**Supplemental Figure 3. The change of urinary AQP2 and the change of body weight in AQP2 responders and non-responders.**

(A) Urinary AQP2 at baseline and at 6 h post-TVP administration are shown on the Y-axis. The solid lines show the change of responders and the dotted lines show the change of non-responders. (B) The maximum decrease in body weight after tolvaptan administration in uAQP2 responders and uAQP2 non-responders are shown on the Y-axis. The box charts for the Y-axis indicate the median as bold lines in the boxes, 25th and 75th percentiles as boxes, 10th and 90th percentiles as lines for each edge.
Figure 1

(A) Changes in body weight

(B) Changes in urinary volume

(C) Changes in serum Na
Figure 2

AUROC 0.729
(95%CI 0.584-0.875)
Figure 3

(A) R=0.48  
p=0.02

(B) R=0.45  
p=0.03

Soluble CD14 (pg/ml) vs. CRP (mg/dl)
Figure 4

The figure shows a box plot comparing soluble CD14 levels between Non-Responder and Responder groups. The p-value of 0.033 indicates a statistically significant difference between the groups.
Figure 5.

(A) Urinary AQP2 (pg/ml) for Non-Responder and Responder groups, showing a significant difference (p<0.01).

(B) Urinary AQP2/Cr for Non-Responder and Responder groups, also showing a significant difference (p<0.01).
**Table 1 Baseline Characteristics of Patients**

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<table>
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<tbody>
<tr>
<td>Number of patients</td>
<td>52</td>
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<tr>
<td>Sex, Male n(%)</td>
<td>36 (69.2)</td>
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<tr>
<td>Age</td>
<td>65 (29-83)</td>
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<tr>
<td>Etiology (HBV / HCV / NBNC)</td>
<td>9 / 18 / 25</td>
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<tr>
<td>HCC, up to Milan’s Criteria n(%)</td>
<td>23 (44.2)</td>
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<td>Habitual drinker n(%)</td>
<td>22 (42.3)</td>
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<tr>
<td>Child·Pugh score (points)</td>
<td>10 (7-13)</td>
</tr>
<tr>
<td>MELD Score (points)</td>
<td>10.85 (3.92-50.69)</td>
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<tr>
<td>The starting dose of tolvaptan, 3.75mg n(%)</td>
<td>44 (84.6)</td>
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<tr>
<td>The diuretics using together</td>
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<tr>
<td>Furosemide (mg/day)</td>
<td>20 (0-80)</td>
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<tr>
<td>Spironolactone (mg/day)</td>
<td>50 (0-100)</td>
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<td>Albumin (g/dl)</td>
<td>2.8 (1.7-3.6)</td>
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<td>Total bilirubin (mg/dl)</td>
<td>1.7 (0.4-15.5)</td>
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<td>PT·INR</td>
<td>1.3 (1.0-2.2)</td>
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<tr>
<td>Serum Creatinin (mg/dl)</td>
<td>0.83 (0.45-3.04)</td>
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<td>BUN (mg/dl)</td>
<td>18 (6.59)</td>
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<td>Serum Na (mEq/l)</td>
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<tr>
<td>CRP (mg/dl)</td>
<td>0.87 (0.02-19.54)</td>
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<td>WBC (/mm3)</td>
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<td>NLR</td>
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<td>Urine Osmolality (mOsm/l)</td>
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<td>NH3 (mg/dl)</td>
<td>73 (19-294)</td>
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<td>AVP (pg/ml) $</td>
<td>2.2 (0.5-13.5)</td>
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<tr>
<td>Urine Na (mEq/l)</td>
<td>64 (9-217)</td>
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<td>Urine K (mEq/l)</td>
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<td>Urine BUN (mEq/l)</td>
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<td>Urine AQP2 (pg/ml)</td>
<td>4.5 (&lt;0.61-39.1)</td>
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<td>Urine AQP2 / Cr ratio</td>
<td>0.0518 (0-0.359)</td>
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<td>soluble CD14 (pg/ml) $</td>
<td>1838 (984-3190)</td>
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<td>Median administration period (Days)</td>
<td>36 (7-976)</td>
</tr>
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</table>

$ The data include only 42 patients  
$ The data include only 37 patients  
HBV Hepatitis B virus, HCV Hepatitis C virus, NBNC Non-HBV Non-HCV virus, MELD The model for End-stage Liver Disease, PT Prothrombin time, BUN Blood urea nitrogen, GFR Glomerular filtration rate, CRP C-reactive protein, WBC White blood cell, NLR Neutrophil / Lymphocyte ratio, AVP Arginine vasopressin, AQP2 Aquaporin-2
Table 2. Pre-treatment predictive factors of TVP treatment (Univariate analysis)

<table>
<thead>
<tr>
<th>Factors</th>
<th>Responders (N= 29)</th>
<th>Non-Responders (N=23)</th>
<th>p value</th>
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<tbody>
<tr>
<td>Age</td>
<td>64 (27-83)</td>
<td>64.5 (15-90)</td>
<td>0.68*</td>
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<tr>
<td>Sex (Male / Female)</td>
<td>22 / 7</td>
<td>14 / 9</td>
<td>0.14**</td>
</tr>
<tr>
<td>Etiology (HBV/HCV/NBNC)</td>
<td>4 / 12 / 13</td>
<td>5 / 6 / 12</td>
<td>0.76**</td>
</tr>
<tr>
<td>HCC (up to milan’s Criteria) (Yes/No)</td>
<td>13 / 16</td>
<td>10 / 13</td>
<td>0.67**</td>
</tr>
<tr>
<td>Child-Pugh Score</td>
<td>9.5 (7-13)</td>
<td>10 (7-12)</td>
<td>0.75*</td>
</tr>
<tr>
<td>MELD Score</td>
<td>10.85 (5.39-50.69)</td>
<td>10.74 (3.92-23.88)</td>
<td>0.70*</td>
</tr>
<tr>
<td>Furosemide</td>
<td>30 (0-80)</td>
<td>20 (0-80)</td>
<td>0.58*</td>
</tr>
<tr>
<td>Spironolactone</td>
<td>50 (0-100)</td>
<td>50 (0-100)</td>
<td>0.42*</td>
</tr>
<tr>
<td>Albumin (g/dl)</td>
<td>2.7 (1.9-3.6)</td>
<td>2.8 (1.7-3.5)</td>
<td>0.74*</td>
</tr>
<tr>
<td>Total bilirubin (mg/dl)</td>
<td>1.8 (0.5-15.5)</td>
<td>1.7 (0.4-10.5)</td>
<td>0.67*</td>
</tr>
<tr>
<td>PT-INR</td>
<td>1.31 (1.01-2.21)</td>
<td>1.36 (1.00-2.07)</td>
<td>0.89*</td>
</tr>
<tr>
<td>Creatinin (mg/dl)</td>
<td>0.85 (0.45-1.97)</td>
<td>0.82 (0.60-3.04)</td>
<td>0.81*</td>
</tr>
<tr>
<td>BUN (mg/dl)</td>
<td>18 (6-53)</td>
<td>20 (8-59)</td>
<td>0.26*</td>
</tr>
<tr>
<td>UA (mg/dl)</td>
<td>5.5 (3.0-16.0)</td>
<td>6.1 (2.7-11.0)</td>
<td>0.66*</td>
</tr>
<tr>
<td>Estimate GFR</td>
<td>72.2 (28.7-180.5)</td>
<td>66.5 (12.8-106.7)</td>
<td>0.53*</td>
</tr>
<tr>
<td>Serum Na (mEq/l)</td>
<td>137 (122-146)</td>
<td>134 (130-142)</td>
<td>0.13*</td>
</tr>
<tr>
<td>CRP (mg/dl)</td>
<td>0.74 (0.02-10.35)</td>
<td>1.86 (0.05-19.54)</td>
<td>0.02*</td>
</tr>
<tr>
<td>WBC (/mm3)</td>
<td>3750 (1100-12700)</td>
<td>4150 (2000-20000)</td>
<td>0.57*</td>
</tr>
<tr>
<td>NLR</td>
<td>3.00 (0.76-17.00)</td>
<td>4.49 (1.41-30.33)</td>
<td>0.01*</td>
</tr>
<tr>
<td>Serum Osmolality (mOsm/l)</td>
<td>281 (264-295)</td>
<td>278.5 (265-295)</td>
<td>0.33*</td>
</tr>
<tr>
<td>Urine Osmolality (mOsm/l)</td>
<td>432 (145-889)</td>
<td>441 (171-794)</td>
<td>0.62*</td>
</tr>
<tr>
<td>NH3 (mg/dl)</td>
<td>78.5 (19-294)</td>
<td>65.5 (34-257)</td>
<td>0.70*</td>
</tr>
<tr>
<td>AVP (pg/ml) §</td>
<td>1.6 (0.3-13.5)</td>
<td>2.5 (0.9-4.9)</td>
<td>0.22*</td>
</tr>
<tr>
<td>Urine Na (mEq/l)</td>
<td>62.5 (22-217)</td>
<td>65.0 (9-167)</td>
<td>0.72*</td>
</tr>
<tr>
<td>Urine Na/K Ratio</td>
<td>3.61 (0.76-19.73)</td>
<td>2.37 (0.19-6.27)</td>
<td>0.12*</td>
</tr>
<tr>
<td>Urine BUN (mEq/l)</td>
<td>113 (483-923)</td>
<td>599 (355-1345)</td>
<td>0.06*</td>
</tr>
<tr>
<td>Urine AQP2 (pg/ml)</td>
<td>2.56 (&lt;0.61-16.2)</td>
<td>6.22 (&lt;0.61-39.2)</td>
<td>0.03*</td>
</tr>
<tr>
<td>Urine AQP2 / Cr ratio</td>
<td>0.034 (0.164)</td>
<td>0.061 (0.021-0.359)</td>
<td>0.04*</td>
</tr>
<tr>
<td>sCD14 (pg/ml) §§</td>
<td>2182(1147-3190)</td>
<td>1607(983-2899)</td>
<td>0.04*</td>
</tr>
</tbody>
</table>

* Statistically analyzed by Mann-Whitney U test  ** Statistically analyzed by Fisher’s exact test
§ The data include only 42 patients  §§ The data include only 37 patients

HBV Hepatitis B virus, HCV Hepatitis C virus, NBNC Non-HBV Non-HCV virus, MELD The model for End-stage Liver Disease, PT Prothrombin time, BUN Blood urea nitrogen, GFR Glomerular filtration rate, CRP C-reactive protein, WBC White blood cell, NLR Neutrophil / Lymphocyte ratio, AVP Arginine vasopressin, AQP2 Aquaporin-2
Table 3. Pre-treatment predictive factors of TVP treatment (Multivariate analysis)

<table>
<thead>
<tr>
<th>Factors</th>
<th>Thresholds</th>
<th>OR</th>
<th>95%CI</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CRP</td>
<td>≧ 0.9 mg/dl</td>
<td>0.179</td>
<td>0.039-0.818</td>
<td>0.026</td>
</tr>
<tr>
<td>NLR</td>
<td>≧ 4.0</td>
<td>0.439</td>
<td>0.093-2.060</td>
<td>0.296</td>
</tr>
<tr>
<td>uBUN</td>
<td>≧ 550 mEq/l</td>
<td>0.356</td>
<td>0.076-1.660</td>
<td>0.189</td>
</tr>
<tr>
<td>Urinary AQP2</td>
<td>≧ 4.5 pg/ml</td>
<td>0.533</td>
<td>0.102-2.790</td>
<td>0.359</td>
</tr>
</tbody>
</table>

*Statistically analyzed by logistic regression analysis

CRP: C-reactive protein, NLR: Neutrophil / Lymphocyte ratio, uBUN: Urinary blood urea nitrogen, AQP2: Aquaporin-2, OR: Odds ratio, CI: Confidence interval