Serum copeptin and zinc-α2-glycoprotein levels are novel biomarkers of tolvaptan treatment in decompensated cirrhotic patients with ascites.

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Research article

Keywords: copeptin, zinc-α2-glycoprotein (ZAG), tolvaptan, liver cirrhosis, ascites

Posted Date: October 6th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-52252/v2

Abstract

Background: The efficacy of tolvaptan, an orally active vasopressin V2-receptor antagonist, has recently been reported in patients with massive ascites unresponsive to conventional diuretics. However, the effect of tolvaptan varies among patients. Recently, the prognostic role of tolvaptan response in decompensated liver cirrhosis (LC) has started to attract more attention. Our aim is to elucidate predictive factors using serum copeptin (vasopressin precursor), zinc-α2-glycoprotein (ZAG), cystatin C (renal biomarker), neutrophil gelatinase-associated lipocalin (NGAL) and liver-type fatty acid-binding protein (L-FABP) that portend a good response to tolvaptan in LC patients with ascites.

Methods: We enrolled 113 LC patients and divided into tolvaptan treatment group and non-treatment group. Tolvaptan (3.75 or 7.5 mg/day) was administrated to 38 LC patients with ascites and a follow-up assessment occurred after a 7-day tolvaptan treatment regimen.

Results: We revealed predictive ability for kidney and/or liver damage in serum copeptin, ZAG, cystatin C, NGAL and L-FABP levels in all patients. Post 7-day tolvaptan treatment, 19 patients lost more than 1.5 kg of their body weight (Responders), while 19 patients did not change their body weight significantly (Non-responders). Basal blood urea nitrogen (BUN) (p=0.0014), serum copeptin (p=0.0265) and serum ZAG levels (p=0.0142) were significantly higher in the Non-responders. BUN (odds ratio 7.43, p=0.0306), copeptin (odds ratio 9.12, p=0.0136) and ZAG (odds ratio 7.43, p=0.0306) were determined to be predictive factors of drug responsiveness using multivariate logistic regression analysis.

Conclusion: Serum BUN, copeptin and ZAG levels predict patient response to tolvaptan even when measured prior to treatment.

Background

Arginine vasopressin (AVP) is a potent antidiuretic hormone in the human body. Despite the clinical relevance of AVP in maintaining fluid balance and vascular tone, measurement of the mature form of AVP is difficult due to small size (9 amino acids), short half-life, and its ability to bind platelets [1]. Copeptin, a 39-amino acid glycopeptide that comprises the C-terminal part of the AVP precursor, was found to be a stable and sensitive surrogate marker for AVP release [2]. Current reports have demonstrated that a high serum copeptin concentration predicts survival hospitalized liver cirrhosis (LC) patients, independent of liver-specific scoring systems [3], suggesting that copeptin has the potential to be a biomarker of disease progression and prognosis in LC [4]. Zinc-alpha-2-glycoperotein (ZAG) is a 41-kDa glycoprotein synthesized by epithelial cells and adipocytes and plays a role in lipid metabolism, cell cycling and cancer progression. Serum ZAG levels has been reported as a biomarker of renal injury and can predict mortality in hemodialysis patients [5]. However, these changes in serum ZAG levels have not been reported in LC patients with ascites.

There are several established markers of renal function, such as serum cystatin C, neutrophil gelatinase-associated lipocalin (NGAL), and liver-type fatty acid binding protein (L-FABP). Urea NGAL is established

as a clinical biomarker for acute renal injury [6] based on NGAL function of a kidney protector [7]. Urine L-FABP levels derived from proximal tubular epithelial cells are elevated in renal tubular injury episodes and are therefore used as an established marker of several kidney diseases, including acute kidney injury and chronic kidney diseases [8]. We have reported that serum NGAL and serum L-FABP levels are prognostic factors for survival in chronic liver diseases [9,10].

Ascites is the most frequent complication of LC and refractory ascites has a negative prognostic implication in the natural progression of LC. Tolvaptan is a novel, orally administered, selective vasopressin V_2-receptor antagonist that downregulates the expression of aquaporin-2 in the renal collecting duct. Current research has uncovered a promising role for tolvaptan as an add-on treatment in patients with hepatic ascites resistant to furosemide and/or spironolactone, as it is able to decrease body weight and alleviate edema [11,12]. However, the effect of tolvaptan is highly variable among patients with approximately one half of the patient cohort responding to the drug [11]. Blood urea nitrogen (BUN) [11] and C-reactive protein (CRP) [12] are recognized for differentiating diagnoses between Responders and Non-responders for tolvaptan, but these factors are insufficient, thus additional biomarkers are needed. Recently, in addition to the aforementioned studies, several studies have also reported Responders to tolvaptan led to improvement of long-term survival rates in cirrhotic patients with ascites [13,14]. Therefore, the aim of this study is to assess the correlation between the efficacy of tolvaptan and treatment related factors, including serum copeptin, ZAG, cystatin C, NGAL and L-FABP levels in LC patients with ascites.

Methods

Human samples

The study protocol was approved by the Clinical Research Ethics Review Committee of Mie University Hospital. This study was performed retrospectively on stored samples, and patients could opt out of their data. In this study, we enrolled 113 LC patients (69 males and 44 females) with a median age of 68.0 (60.0-75.0) years who could be analyzed between November 2013 and September 2016 at Mie University hospital. Patients positive for hepatitis B surface antigen were diagnosed HBV infection, whereas those positive for anti-hepatitis C virus (HCV) were diagnosed HCV infection. Alcoholic cirrhosis defined as the presence of alcohol consumption >60 g/day. Nonalcoholic steatohepatitis was diagnosed based on pathological findings or fatty liver without any other evident causes of chronic liver diseases (viral, autoimmune, genetic, etc.). We divided the 113 LC patients into three groups: without ascites group (n=46), with ascites non-treatment group (n=22) and with ascites treatment group (n=45) (Table 1). LC was diagnosed based on morphologic changes of the liver such as hypertrophy of the left lateral and caudate lobes, or atrophy of the right posterior hepatic lobe on ultrasonography and through blood tests, and/or computed tomography, magnetic resonance imaging, FibroScan (Echosens, French) results, and esophageal varix by endoscopy, as is the general protocol. FibroScan cannot be used in individuals with ascites, therefore, it was performed for assessing liver fibrosis mainly in chronic hepatitis or early

cirrhosis. The definition of idiopathic portal hypertension/portal-sinusoidal vascular disorder was based on the absence of characteristic cirrhosis and known causes of liver disease. Decompensated cirrhosis was diagnosed as it presents with a series of clinical and laboratory signs, e.g. ascites, encephalopathy, gastrointestinal bleeding, thrombocytopenia or hypoalbuminemia. HCC was diagnosed based on histological findings or typical imaging characteristics. Body mass index (BMI) was calculated as weight (kg)/height (m) squared. BMI was not adjusted for ascites. We provided body composition analysis by measuring the psoas muscle index (PMI) due to the limitations of BMI in patients with ascites. Using cross-sectional CT at the level of transverse process of lumbar vertebra L3, the bilateral psoas muscle area was identified. PMI (cm^2/m^2) was defined by normalizing psoas muscle area (cm^2)/height (m) squared. Patients who had other malignancies within the past 3 years, spontaneous bacterial peritonitis, hepatic encephalopathy (coma scale score ≥ II), heart failure (the New York Heart Association defined category ≥class II), human immunodeficiency virus infection, pregnancy, or psychiatric problems were deemed to be unsuitable for clinical study.

Algorithm for treatment of ascites and definition of responder and non-responder

As a general rule, the follow-up examinations included routine physical examinations and biochemical tests and diagnostic imagining studies including ultrasonography. All treatments of ascites were performed following the Japanese practical guidelines for LC in 2015 as possible [15]. Patients with grade 1 mild ascites do not need diuretics and a low sodium diet. For patients with grade 2 moderate ascites, treatment begins with the administration of spironolactone at 25-50 mg /day, then, in the absence of an effect, furosemide is added at 20 mg/day. Tolvaptan was recommended adding on other diuretics including 25-50 mg/day spironolactone and/or 20-40 mg/day furosemide, especially in patients with sustained hyponatremia.

We divided the patients into two tolvaptan treatment groups: Responders and Non-responders. The Non-responder group was defined as patients with weight loss of <1.5 kg/week after receiving tolvaptan or performing paracentesis within the first week [12]. All patients continued to take the same prescribed doses of furosemide and spironolactone within the first week [16].

Serum preparation

In the without ascites group and with ascites non-treatment group, serum samples were collected when patients showed up at the hospital. In the with ascites treatment group, serum collection was immediately before the administration of tolvaptan. Albumin (g/dL), total bilirubin (mg/dL), sodium (mEq/L), creatinine (mg/dL), BUN (mg/dL), CRP (mg/dL), α-fetoprotein (AFP; ng/mL) and des-γ-carboxy prothrombin (DCP; mAU/mL) were measured. Serum were kept at -80℃ until copeptin (pmol/L), ZAG (μg/dL), cystatin C (mg/dL), NGAL (ng/mL) and L-FABP (ng/mL) measurements, using an automated copeptin immunofluorescent assay kit (Thermo Fisher Scientific Inc., Tokyo) [2], ZAG enzyme-linked immunosorbent assay (ELISA) kit (BioVendor, Czech Republic), cystatin C ELISA kit (R&D systems, Minneapolis, MN), NGAL ELISA kit (R&D systems, Minneapolis, MN) and high-sensitivity human L-FABP

ELISA kit (CMIC Holdings Co., Ltd., Tokyo), respectively. The Child-Pugh score, albumin-bilirubin (ALBI) score [17], the fibrosis index based on 4 factors (FIB-4) [18], model for end-stage liver disease (MELD) score, MELD-Na score and estimated glomerular filtration rate (eGFR) with Cockcroft and Modification of Diet in Renal Disease (MDRD) formula were calculated.

Statistical analysis

All data are expressed as median and range. Data were analyzed using the Mann-Whitney *U* test in two groups and one-way analysis of variance for comparison of continuous variables. The relationship between serum copeptin, ZAG, cystatin C, NGAL, L-FABP levels and clinical data were examined using Spearman's rank correlation coefficient. For each continuous variable, the optimal cutoff value that maximized the sum of sensitivity and specificity was selected using receiver operating characteristic (ROC) analysis for survival. A logistic regression analysis was utilized for the multivariate analysis in order to evaluate the relationship between effect of tolvaptan and clinical data. Only variables deemed to be significant ($p<0.1$) in the univariate analysis were included in the subsequent multivariate analysis. The statistical analyses were performed using JMP software program (SAS Institute, Cary, NC, USA) for univariate and multivariate logistic regression analysis. Differences were considered to be significant at $p<0.05$.

Results

Clinical characteristics of patients with or without tolvaptan

Cohort creation were summarized in the patient flow (supplementary Figure 1). In this study, we divided the 113 LC patients into 3 groups : without ascites group, with ascites non-treatment group or with ascites treatment group. Table 1 shows the comparison of baseline clinical characteristics and laboratory variables between non-treatment group and treatment group. In the treatment group, AST, total bilirubin, CRP, the Child-Pugh score, ALBI score and FIB4-index were significantly increased, while serum albumin and sodium levels were reduced, suggesting that patients treated with tolvaptan demonstrated more advanced liver diseases. In the treatment group, the dose of diuretics drugs (Furosemide and/or Spironolactone) was significantly higher. In contrast, there were no obvious differences in age, gender, bodyweight, BMI, PMI, prevalence of HCC, ALT, prothrombin time, MELD score, MELD-Na score, creatinine, BUN, eGFR (Cockcroft and MDRD-6), copeptin, ZAG, NGAL, L-FABP and cystatin C between the two groups (Table 1).

Correlation of copeptin, ZAG, cystatin C, NGAL and L-FABP to clinical parameters in all patients

The correlations between copeptin, ZAG, cystatin C, NGAL or L-FABP and clinical parameters in LC patients were shown in Table 2. Copeptin was strongly correlated with mainly hepatic function including albumin ($p=0.0007$; Figure 1-D), Child-Pugh score ($p<0.0001$), ALBI score ($p=0.0003$) and CRP ($p=0.0047$; Figure 1-C). In contrast, ZAG was more strongly correlated with renal function including creatinine ($p<0.0001$), BUN ($p<0.0001$) and GFR (MDRD-6) ($p<0.0001$) (Table 2; Figure 1-K and L). In addition, ZAG

was correlated with bodyweight (p=0.0092; Figure 1-G), BMI (p=0.0001; Figure 1-H) and CRP (p<0.0001; Figure 1-I). Cystatin C was significantly correlated with age (p=0.0191), renal parameters (creatinine, BUN and eGFR: p<0.0001), copeptin (p=0.0008), NGAL (p<0.0001) and L-FABP (p<0.0001) (Table 2). NGAL and L-FABP were significantly correlated with indicators of both renal function and hepatic function (Table 2). MELD-Na score significantly correlated with all, copeptin (p=0.0008), ZAG (p=0.001), cystatin C (p<0.0001), NGAL (p<0.0001) and L-FABP (p=0.0003).

Background comparison between Responders and Non-responders to tolvaptan

In this study, we excluded 7 from 45 cases for which tolvaptan efficacy could not be determined because of transferring hospitals, lack of weight data or with other treatment such as albumin transfusion, large volume paracentesis, and cell-free and concentrated ascites reinfusion therapy (Supplementary Figure 1). We enrolled 38 decompensated LC patients with ascites (24 males and 14 females) with a median age of 66.0 (59.5-73.0) years. We divided the 38 patients into two tolvaptan treatment groups: Responders and Non-responders. There were no obvious differences in age, gender, bodyweight, BMI, PMI, presence of HCC, AFP, DCP, dose of diuretic drugs, albumin, total bilirubin, Child-Pugh score, ALBI score, FIB4-index, MELD score/MELD-Na score, creatinine, eGFR, serum sodium, cystatin C, NGAL and L-FABP between the two groups (Table 3; Figure 2-D, E and F). In contrast, BUN, copeptin and ZAG levels were significantly higher in Non-responders when compared to the Responders group (respectively, p=0.0014, p=0.0265, p=0.0142) (Table 3; Figure 2-A, B and C). We further examined except for HCC, because HCC have pro-inflammatory condition. BUN (p=0.015) was significantly higher in Non-responders (n=7) when compared to the Responders (n=8) in patients without HCC. In contrast, copeptin and ZAG levels were not higher in Non-responders in patients without HCC.

Predictors contributing to the effect of tolvaptan in treatment for ascites

We calculated the cutoff values, area under the ROC curve, sensitivity and specificity of BUN, copeptin and ZAG using ROC analysis. The cutoff values ascertained from our analyses of BUN, copeptin and ZAG were 18.3mg/dL, 10.1pmol/L and 32.4 μg/mL, respectively (Table 4). The contribution of HCC, BUN, copeptin and ZAG were evaluated using multivariate logistic regression analysis. We found serum BUN (odds ratio 7.43, p=0.0306), copeptin (odds ratio 9.12, p=0.0136) and ZAG (odds ratio 7.43, p=0.0306) levels to be the significant predictors contributing to the efficacy of tolvaptan in the treatment for ascites; however, presence of HCC was not selected (Table 4).

Discussion

In this study, we revealed for the first time that serum copeptin and ZAG levels were significantly increased in the tolvaptan treatment Non-responders group when compared to the Responders group. In addition, we indicated that serum BUN, copeptin and ZAG levels were independent predictors of the overall response to tolvaptan therapy. We further showed that serum creatinine and cystatin C levels were not predictive of patient response to tolvaptan, thus confirming the findings reported by others [11,12]. In LC, arteriolar vasodilation causes underfilling of the systemic arterial vascular space, and the decrease in the

effective blood volume leads to a decrease in arterial pressure [19]. Consequently, activation of the renin-angiotensin-aldosterone system, sympathetic nervous system and the release of antidiuretic hormone take place as the body attempts to restore normal blood pressure homeostasis [20]. The main physiological function of AVP is antidiuresis, thereby regulating systemic osmotic pressure. Plasma AVP levels are normally regulated by plasma osmotic pressure and have been found to be elevated in LC patients with ascites, as reported by Pérez-Ayuso RM [21]. However, there has been no study to date exploring whether the effect of tolvaptan is related to plasma AVP or pro-AVP (copeptin) levels in ascites patients. The Non-responder group is considered to be in a state of relative vascular underfilling with an increase in BUN and copeptin, thus suggesting intravascular dehydration [11]. Furthermore, serum copeptin also correlated with multiple factors including renal function and CRP, resulting in copeptin was not only a biomarker of renal function. Although NGAL and L-FABP are useful markers than copeptin for acute-on-chronic liver failure [22] and acute kidney injury [23], respectively, this study indicated that serum NGAL and L-FABP were not useful in assessing the efficacy of response to tolvaptan in LC patients with ascites.

Serum ZAG, it has been determined, is increased in acute kidney injury [24] and in minor kidney injury caused by normo-albuminuric diabetic kidney disease – patients presenting with renal insufficiency, but no significant proteinuria [25]. On the other hand, serum cystatin C accurately reflects renal function, while ZAG reflects lipolysis and renal function. Serum ZAG has been expected as a biomarker for cachexia [26]. Indeed, in this study, ZAG correlated with body weight, BMI and CRP. Bellos also reported that body weight loss and elevated CRP were the predictors for Non-responders to tolvaptan [12]. We assumed that association of serum ZAG with multiple factors including renal function, cachexia (relating to body weight and BMI) and/or inflammation (relating to CRP) led to the result.

Based on the evidence outlined above, serum copeptin may reveal the underlying pathological condition of decreased osmotic pressure, intravascular dehydration and inflammation, whereas serum ZAG may reflect minor kidney injury, cachexia and inflammation, suggesting that copeptin and ZAG are independent predictors of patient response to tolvaptan.

Although many patients with LC are complicated by HCC, it is not known whether coexistent HCC linked to response to tolvaptan therapy. Recent large-scale post-marketing surveillance study (n=1111) reported that there was no difference in mean of weight reduction by tolvaptan treatment between patients with and without HCC (p=0.2248) [27]. Both the American and European clinical practice guidelines recommended high-dose diuretic treatment against massive ascites [15]. In contrast, the Japanese guideline recommended additional tolvaptan administration for massive ascites. Together, clinical trials involving a large number of patients are needed to determine the timing of tolvaptan initiation.

This study has several limitations. This was a retrospective and single-center study with a small sample size and a short follow-up period. Therefore, the confidence intervals of the predictors for tolvaptan treatment of ascites were huge due to a small sample size. Further study using a larger cohort, especially many cirrhotic patients without HCC, is required to investigate the interaction of copeptin, ZAG, and BUN

and the possible predictors of patient survival. In conclusion, serum BUN < 18.3 mg/dL, copeptin < 10.1 pmol/L and ZAG < 32.4 μg/ml appear to be good predictors of overall patient response to tolvaptan treatment.

Conclusions

Serum copeptin, ZAG and BUN levels may be used as novel biomarkers to determine overall response to tolvaptan in patients presenting with LC and ascites. Future study is required to develop a new clinical prediction model using serum copeptin, ZGA and BUN that would help clinicians determine the effectiveness of tolvaptan treatment.

Abbreviations

LC, liver cirrhosis; ZAG, zinc-α2-glycoprotein; AVP, arginine vasopressin; NGAL, neutrophil gelatinase-associated lipocalin; L-FABP, liver-type fatty acid-binding protein; HCV, hepatitis C virus; BMI, Body mass index, PMI, Psoas muscle index; BUN, blood urea nitrogen; CRP, C-reactive protein; AFP, α-fetoprotein; DCP, des-γ-carboxy prothrombin; ALBI, albumin-bilirubin; FIB-4, fibrosis index based on 4 factors; MELD, Model for end-stage liver disease; eGFR, estimated glomerular filtration rate; MDRD, Modification of Diet in Renal Disease; ROC, receiver operating characteristic

Declarations

Statement of Ethics

The study protocol was approved by the Clinical Research Ethics Review Committee of Mie University Hospital (approval no. H2019-190). This research comply with the guidelines for human studies and should include evidence that the research was conducted ethically in accordance with the World Medical Association Declaration of Helsinki. The subjects (or their parents or legal guardians) have given their written informed consent.

Conflict of Interest Statement (Competing interests)

Authors does not have any conflict of interests, except Motoh Iwasa received a lecture fee from Otsuka Pharmaceutical Co., Ltd, which manufactures tolvaptan.

Consent to publish; All authors agree to publish.

Availability of data and materials; Department of Gastroenterology and Hepatology, Mie University Graduate School of Medicine

Funding Sources

No external financial support was received.

Author's contributions

RS: statistical analysis, data interpretation and drafting the article; MI: study concept, design, data acquisition, data interpretation and drafting the article; AE: sample analyses, data interpretation and critical revision of the article for important intellectual content; MT: sample analyses; YT: sample collection; TS: data analysis; YT: critical revision of the article for important intellectual content; All authors: approval of the final version of the manuscript

Acknowledgement; There was no acknowledgement.

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Tables

Table 1. Characteristics of 113 liver cirrhotic patients.

Parameters	All	Without ascites	With ascites		
			Non-treatment	Treatment	$p^{\dagger}$
	n = 113	n = 46	n = 22	n = 45	
Age (years)	68.0 (60.0-75.0)	66.5 (58.0-72.8)	74.0 (66.3-80.8)	68.0 (61.0-76.0)	0.1117
Gender, Male : Female	69 : 44	29 : 17	14 : 8	26 : 19	0.6939
Bodyweight (kg)	58.1 (51.3-66.7)	62.3 (51.3-72.7)	57.0 (53.4-61.6)	56.6 (51.0-63.6)	0.7620
Body mass index (kg/m^2)	23.6 (20.8-25.3)	24.0 (22.5-25.8)	22.3 (20.1-25.0)	22.1 (19.5-24.3)	0.7010
Psoas muscle index (cm^2/m^2)	5.6 (4.7-7.0)	6.1 (4.7-7.4)	5.6 (4.8-6.4)	5.2 (4.2-6.2)	0.2651
HCV/HBV/HCV+AL/AL/NASH/others	56/7/4/23/13/10	28/2/0/10/2/4	11/0/1/3/4/3	17/4/3/10/7/4	-
presence of HCC, yes: no	61 : 52	23 : 23	10 : 12	28 : 17	0.6045
Ratio of using diuretic drugs (%)		26	45	100	-
Dose of diuretic drugs (n)					
Tolvaptan (0mg/3.75mg/7.5mg)	68 / 37 / 8	46 / 0 / 0	22 / 0 / 0	0 / 37 / 8	-
Furosemide (0mg/10mg/20mg/40mg/80mg)	62/4/28/14/5	40/0/4/2/0	16/1/4/0/1	6/3/20/12/4	**<0.0001**
Spironolactone (0mg/25mg/50mg/100mg)	55 / 26 / 28 / 4	38 / 4 / 3 / 1	14 / 4 / 3 / 1	3 / 18 / 22 / 2	**<0.0001**
Grade of ascites (1 / 2 / 3)	37 / 22/ 8	0 / 0 / 0	17 / 2 / 3	20 / 20 / 5	-
AST (U/L)	45.0 (31.0-70.0)	37.0 (28.3-54.5)	35.5 (28.0-62.5)	55.0 (42.0-87.0)	**0.0014**
ALT (U/L)	29.0 (17.0-41.0)	28.0 (17.0-41.8)	29.5 (15.0-34.3)	29.0 (23.0-42.0)	0.2421
Albumin (g/dL)	3.2 (2.8-3.7)	3.6 (3.3-4.1)	3.3 (3.0-3.7)	2.9 (2.6-3.1)	**0.0010**
Total Bilirubin (mg/dL)	1.2 (0.7-1.9)	1.1 (0.7-1.4)	0.8 (0.7-1.8)	1.5 (0.8-2.7)	**0.0210**
Prothrombin time (%)	72.0 (60.9-82.4)	75.6 (64.1-84.0)	74.2 (61.5-85.5)	67.4 (55.1-78.4)	0.1297
Child-Pugh score	7.0 (6.0-9.0)	5.5 (5.0-6.8)	7.5 (6.3-9.0)	8.0 (8.0-10.0)	**0.0083**
ALBI score	-1.9 (-2.4- -1.5)	-2.4 (-2.6--1.9)	-2.0 (-2.7--1.3)	-1.5 (-1.8--1.2)	**0.0004**
FIB4-index	6.3 (3.8-9.3)	5.2 (3.5-7.9)	5.0 (3.7-8.4)	8.0 (5.5-10.6)	**0.0343**
MELD score	16.0 (14.6-18.2)	15.0 (14.0-16.1)	16.5 (15.8-20.0)	16.9 (15.0-19.3)	0.6643
MELD-Na score	18.3 (15.5-21.5)	15.6 (13.5-17.4)	17.6 (16.5-21.7)	20.3 (18.4-23.5)	0.0557
Creatinine (mg/dL)	0.8 (0.6-1.0)	0.7 (0.6-0.8)	0.9 (0.8-1.1)	0.8 (0.7-1.0)	0.2143
BUN (mg/dL)	15.5 (11.7-22.0)	14.0 (10.0-18.0)	18.0 (13.5-27.2)	18.5 (13.0-25.0)	0.6594
eGFR (mL/min/1.73m^2)	69.0 (59.0-104.4)	75.1 (59.3-93.6)	58.8 (34.4-74.5)	61.1 (46.7-87.7)	0.4999
GFR (MDRD-6) (mL/min)	84.3 (70.4-126.4)	99.6 (80.4-128.2)	75.5 (53.0-95.0)	77.2 (54.0-95.7)	0.5887
Serum sodium (mEq/L)	138 (134-140)	140 (139-141)	138 (136-139)	134 (131-138)	**0.0043**
CRP (mg/dL)	0.3 (0.1-1.3)	0.1 (0.0-0.3)	0.2 (0.1-1.0)	1.2 (0.4-3.4)	**<0.0001**
Copeptin (pmol/L)	6.6 (3.6-14.2)	4.2 (2.6-6.7)	8.0 (5.2-15.2)	10.7 (5.4-19.7)	0.5002
ZAG (μg/mL)	29.2 (24.3-37.6)	27.4 (22.5-32.0)	37.9 (26.9-45.6)	32.1 (24.7-41.1)	0.1797
NGAL (ng/mL)	61.0 (30.1-104.2)	30.3 (18.4-53.3)	88.9 (43.9-114.5)	82.9 (54.8-149.8)	0.6595
Cystatin C (mg/dL)	1.1 (0.9-1.3)	1.0 (0.8-1.2)	1.2 (1.0-1.5)	1.1 (0.9-1.4)	0.2854
L-FABP (ng/mL)	8.1 (3.8-13.8)	5.3 (2.2-13.3)	8.4 (3.8-10.8)	9.7 (5.5-19.3)	0.2119

† Mann–Whitney *U* test (Non-treatment vs. Treatment). HCV, hepatitis C virus; HBV, hepatitis B virus; AL, alcohol; NASH, nonalcoholic steatohepatitis; HCC, hepatocellular carcinoma; AST, aspartate aminotransferase; ALT, alanine

aminotransferase; ALBI, albumin-bilirubin; FIB4-index, fibrosis-4; MELD, Model for End-Stage Liver Disease; BUN, blood urea nitrogen; eGFR, estimated glomerular filtration rate; MDRD-6, the 6-variable Modification of Diet in Renal Disease; CRP, C-reactive protein; ZAG, zinc-α2-glycoprotein; NGAL, neutrophil gelatinase-associated lipocalin; L-FABP, Liver-type fatty acid–binding protein. All data are expressed as median (first quartile-third quartile).

Table 2. Correlation between serum copeptin, ZAG, cystatin C, NGAL, L-FABP levels and clinical parameters in 113 liver cirrhotic patients.

ter	Copeptin		ZAG		Cystatin C		NGAL		L-FABP	
	r	$p^{※}$	r	$p^{※}$	r	$p^{※}$	r	$p^{※}$	r	$p^{※}$
	-0.0127	0.8930	0.1691	0.0734	0.2202	**0.0191**	0.1902	**0.0436**	0.0945	0.3194
	-0.0623	0.5120	0.1474	0.1191	-0.0729	0.4429	-0.0762	0.4223	0.1380	0.1448
ht	-0.0417	0.6683	-0.2497	**0.0092**	-0.1195	0.2182	-0.0907	0.3507	-0.1156	0.2336
	-0.0504	0.6100	-0.3675	**0.0001**	-0.0453	0.6460	-0.0796	0.4197	-0.1695	0.0839
	-0.1605	0.1070	-0.0610	0.5425	-0.1211	0.2252	-0.0484	0.6293	-0.0676	0.4998
	0.1947	**0.0388**	-0.2462	**0.0086**	-0.0894	0.3464	0.0226	0.8120	0.3504	**0.0001**
	0.0521	0.5835	-0.1792	0.0575	-0.0647	0.4959	-0.0707	0.4567	0.4015	**< 0.0001**
	0.1580	0.0947	-0.3090	**0.0009**	-0.1947	**0.0388**	-0.0392	0.6804	-0.0671	0.4800
	-0.3152	**0.0007**	-0.0022	0.9819	-0.1280	0.1766	-0.4398	**< 0.0001**	-0.2312	**0.0137**
bin	-0.1141	0.2311	0.2386	**0.0113**	-0.1582	0.0957	-0.0752	0.4308	-0.0918	0.3357
e	0.2197	**0.0199**	0.4322	**< 0.0001**	0.5714	**< 0.0001**	0.4085	**< 0.0001**	0.3497	**0.0001**
	0.2575	**0.0059**	0.5037	**< 0.0001**	0.5074	**< 0.0001**	0.3703	**< 0.0001**	0.4430	**< 0.0001**
	-0.2490	**0.0078**	-0.3451	**0.0002**	-0.6086	**< 0.0001**	-0.4481	**< 0.0001**	-0.2788	**0.0028**
)RD-	-0.3018	**0.0012**	-0.3731	**< 0.0001**	-0.6077	**< 0.0001**	-0.4949	**< 0.0001**	-0.3412	**0.0002**
	-0.1200	0.2181	-0.1232	0.2062	-0.0285	0.7711	-0.2553	**0.0080**	-0.1790	0.0650
	0.2701	**0.0047**	0.3677	**<0.0001**	0.0518	0.5944	0.4291	**< 0.0001**	0.2281	**0.0176**
	-	-	0.1969	**0.0366**	0.3104	**0.0008**	0.3182	**0.0006**	0.1709	0.0703
	0.1969	**0.0366**	-	-	0.2701	**0.0038**	0.3634	**< 0.0001**	0.1735	0.0662
C	0.3104	**0.0008**	0.2701	**0.0038**	-	-	0.4913	**< 0.0001**	0.4468	**< 0.0001**
	0.3182	**0.0006**	0.3634	**< 0.0001**	0.4913	**< 0.0001**	-	-	0.3105	**0.0008**
	0.1709	0.0703	0.1735	0.0662	0.4468	**< 0.0001**	0.3105	**0.0008**	-	-
h	0.3819	**<0.0001**	-0.0271	0.7754	0.1468	0.1208	0.3876	**< 0.0001**	0.2143	**0.0227**
e	0.3366	**0.0003**	-0.0921	0.3322	0.0656	0.4901	0.3750	**< 0.0001**	0.2078	**0.0272**
ore	0.3057	**0.0010**	0.3338	**0.0003**	0.5595	**< 0.0001**	0.4498	**< 0.0001**	0.2839	**0.0024**
	0.3187	**0.0008**	0.3135	**0.0010**	0.4342	**< 0.0001**	0.5188	**< 0.0001**	0.3450	**0.0003**

※ Spearman's rank correlation coefficient. BMI, body mass index; PMI, psoas muscle index; AST, aspartate aminotransferase; ALT, alanine aminotransferase; BUN, blood urea nitrogen; eGFR, estimated glomerular filtration rate; MDRD-6, the 6-variable Modification of Diet in Renal Disease; CRP, C-reactive protein; ZAG, zinc-α2-glycoprotein;

NGAL, neutrophil gelatinase-associated lipocalin; L-FABP, Liver-type fatty acid–binding protein; ALBI, albumin-bilirubin; MELD, Model for End-Stage Liver Disease.

Table 3. Characteristics of patients treated with Tolvaptan.

Parameters	Responders n = 19	Non-responders n = 19	$p^{\dagger}$
Age (years)	65.0 (60.5-74.0)	68.0 (58.0-73.5)	0.7589
Gender, Male : Female	13 : 6	11 : 8	0.5795
Bodyweight (kg)	61.2 (55.6-64.4)	57.0 (50.9-63.1)	0.4229
Body mass index (kg/m^2)	21.9 (19.3-24.8)	22.5 (19.2-24.7)	0.8315
Psoas muscle index (cm^2/m^2)	5.7 (4.5-7.1)	5.3 (4.6-5.9)	0.5534
HCV/HBV/HCV+AL/AL/NASH/others	7/0/2/6/2/2	5/4/1/4/4/1	-
presence of HCC, yes: no	11 : 8	12 : 7	0.7862
Dose of diuretic drugs			
Tolvaptan (3.75 mg/7.5 mg)	15 / 4	17 / 2	0.6976
Furosemide (mg)	20 (5-35)	20 (20-40)	0.8217
Spironolactone (mg)	50 (25-50)	25 (25-50)	0.2548
Albumin (g/dL)	2.9 (2.6-3.1)	2.8 (2.7-3.2)	0.8490
Total Bilirubin (mg/dL)	1.8 (1.2-3.1)	1.4 (0.8-2.3)	0.1361
Child-Pugh score	8.0 (8.0-10.5)	8.0 (7.5-10.0)	0.3679
ALBI score	-1.4 (-1.8- -1.1)	-1.5 (-1.8 - -1.3)	0.7371
FIB4-index	7.8 (6.3-10.1)	8.3 (5.0-10.4)	0.9534
MELD score	16.6 (15.0-19.5)	17.4 (15.8-20.0)	0.3655
MELD-Na score	20.3 (18.9-22.5)	21.3 (18.0-23.7)	0.9302
Creatinine (mg/dL)	0.8 (0.6-0.9)	0.9 (0.7-1.1)	0.2932
BUN (mg/dL)	13.0 (10.0-19.0)	23.0 (18.8-27.5)	**0.0014**
eGFR ($mL/min/1.73m^2$)	69.4 (50.2-81.8)	58.2 (45.4-76.6)	0.2093
GFR (MDRD-6) (mL/min)	82.8 (60.1-102.7)	60.2 (51.8-88.6)	0.0904
Serum sodium (mEq/L)	134 (132-137)	135 (132-139)	0.2909
CRP (mg/dL)	1.2 (0.4-2.7)	1.9 (0.4-3.8)	0.5018
Copeptin (pmol/L)	6.6 (4.0-14.0)	14.0 (9.0-27.2)	**0.0265**
ZAG (μg/mL)	27.4 (23.1-33.2)	37.4 (32.8-44.9)	**0.0142**
NGAL (ng/mL)	95.1 (59.5-162.0)	82.9 (61.2-140.6)	1.0000
Cystatin C (mg/dL)	1.1 (0.9-1.3)	1.1 (0.8-1.4)	0.9534
L-FABP (ng/mL)	8.5 (4.4-17.5)	11.5 (8.1-17.9)	0.2429
AFP (ng/mL)	9 (5-335)	8 (3-67)	0.4362
DCP (mAU/mL)	498 (39-8039)	2816 (157-9950)	0.5065

† Mann–Whitney *U* test, HCV, hepatitis C virus; HBV, hepatitis B virus; AL, alcohol; NASH, nonalcoholic steatohepatitis; HCC, hepatocellular carcinoma; ALBI, albumin-bilirubin; FIB4-index, fibrosis-4; MELD, Model for End-Stage Liver Disease; BUN, blood urea nitrogen; eGFR, estimated glomerular filtration rate; MDRD-6, the 6-variable Modification of Diet in Renal Disease; CRP, C-reactive protein; ZAG, zinc-α2-glycoprotein; NGAL, neutrophil gelatinase-associated lipocalin; L-FABP, Liver-type fatty acid–binding protein; AFP, α-fetoprotein; DCP, Des-γ-carboxy prothrombin. Responders were defined as decompensated liver cirrhosis patients in whom tolvaptan was an effective treatment for ascites. All data are expressed as median (first quartile-third quartile).

Table 4. Predictors contributing to the effect of Tolvaptan in the treatment for ascites

ictors	cutoff	ROCAUC	Sensitivity	Specificity	Univariate analysis			Multivariate analysis		
					OR	95%CI	$p^{§}$	OR	95%CI	$p^{§}$
	18.3	0.8047	79	74	10.50	2.54 → 53.39	0.0009	7.43	1.20 → 64.69	**0.0306**
ptin	10.1	0.7119	68	74	6.07	1.57 → 27.02	0.0083	9.12	1.54 → 91.14	**0.0136**
	32.4	0.7341	79	74	10.50	2.54 → 53.39	0.0009	7.43	1.20 → 64.69	**0.0306**
	-	-	-	-	1.24	0.34 → 4.69	0.7399	-	-	-

ROCAUC, receiver operating characteristic area under the curve.

$p^{§}$; Logistic regression analysis; OR, odds ratio; 95%CI, 95% confidence interval

BUN, blood urea nitrogen; ZAG, zinc-α2-glycoprotein; HCC, hepatocellular carcinoma.

Figures

Figure 1

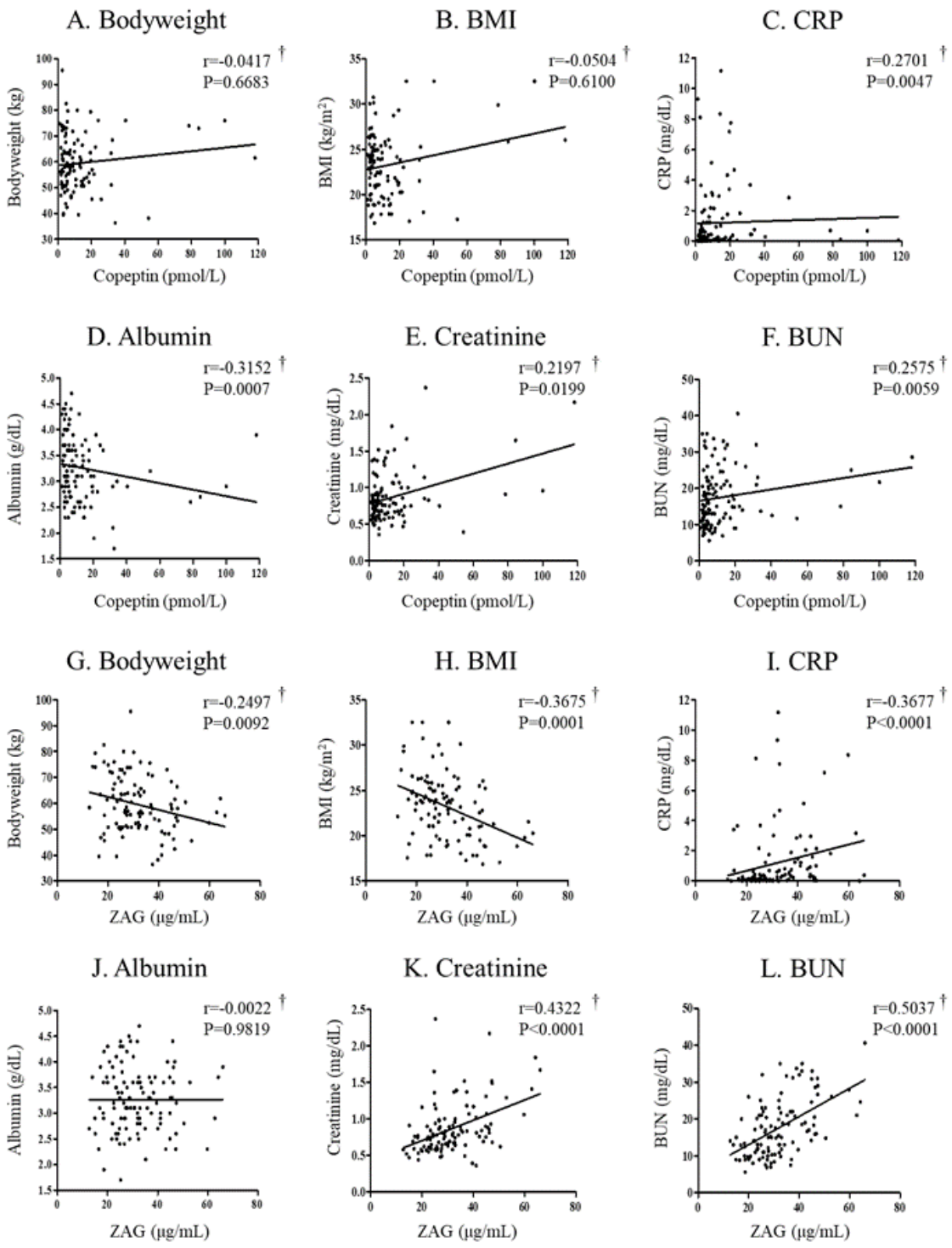

Figure 1

Serum copeptin levels were correlated with liver and kidney function in patients with cirrhosis. Serum ZAG levels were correlated with bodyweight, BMI and kidney function in patients with cirrhosis. Correlation between serum copeptin levels and Bodyweight (A), BMI (B), CRP (C), albumin (D), creatinine (E) or BUN (F). Correlation between serum ZAG levels and Bodyweight (G), BMI (H), CRP (I), albumin (J), creatinine

(K) or BUN (L). ZAG, zinc-α2-glycoprotein; BMI, body mass index; CRP, C-reactive protein; BUN, blood urea nitrogen

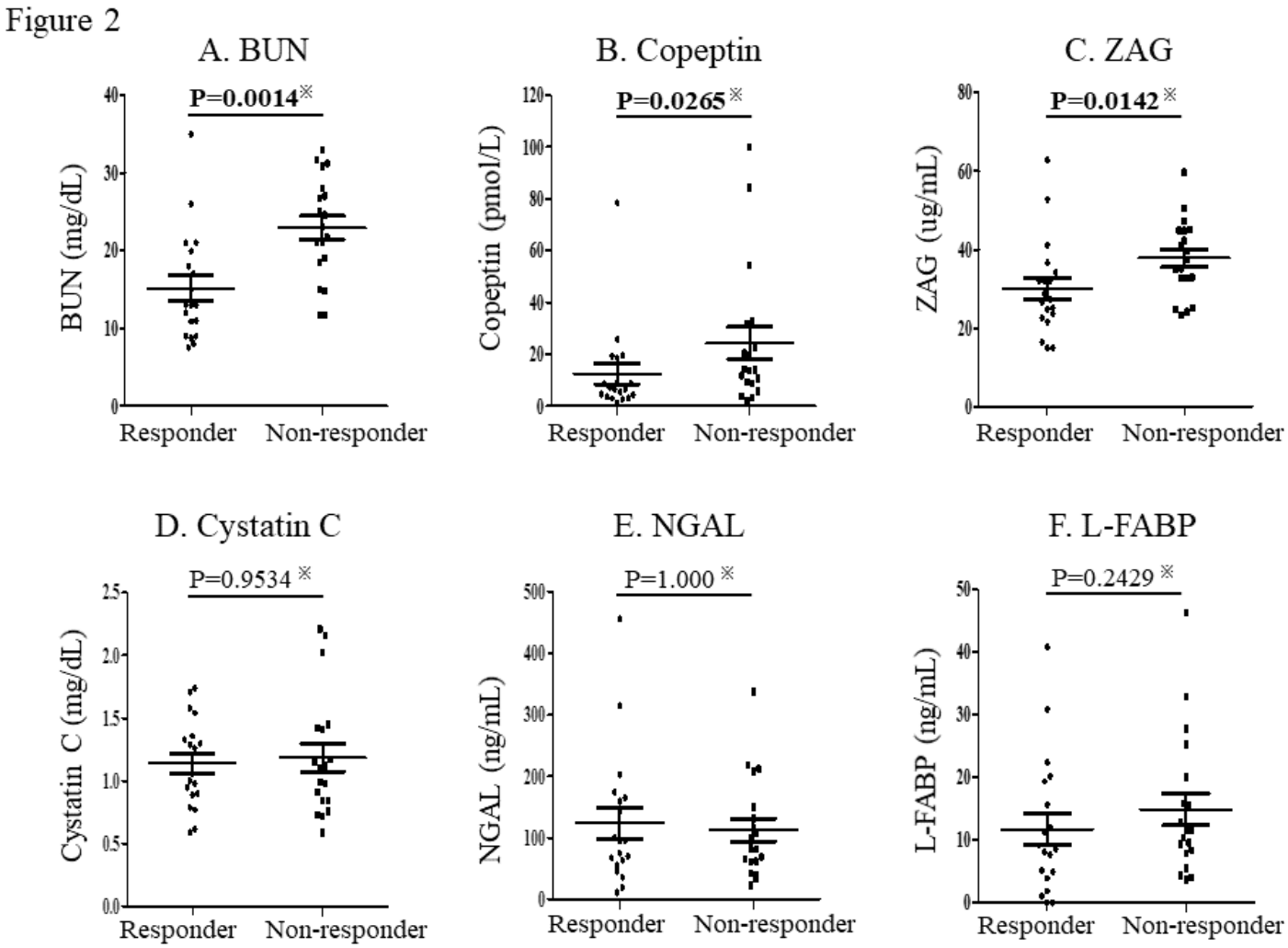

Figure 2

Predictors contributing to the effect of tolvaptan in treatment for ascites. Serum BUN (A), copeptin (B), ZAG (C), cystatin C (D), NGAL (E) and L-FABL (F) levels in the Responder and Non-responder to tolvaptan. BUN, blood urea nitrogen; ZAG, zinc-α2-glycoprotein; NGAL, neutrophil gelatinase-associated lipocalin; L-FABP, liver-type fatty acid-binding protein

Supplementary Files

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- Suppl.1Patientflow.jpg