

Damian Garcia-Olmo, M.D., Ph.D.
Editor-in-Chief
World Journal of Gastroenterology

Dear Dr. Garcia-Olmo,

We thank the referees for their careful review of our manuscript and for giving us useful comments that have helped in its revision. We have revised the manuscript, **ID 36146**, in accordance to the reviewers' comments and are looking forward to its publication in your most esteemed journal, the **World Journal of Gastroenterology**.

Our response for Reviewer 1.

Thank you for your detailed comments that have helped us to improve our manuscript.

Comment 1: Sample size in this study is small and how to calculate the required number is not very clear in section "Statistical analysis".

Reply: Sample size was based on previous studies. To make this point clearer, we have added the following to the Discussion (p. 10, lines 18-28):

“Our estimation of clinical effects using change in body weight after a combination diuretic therapy for a week was based on a previous study [11]. The change of body weight after 1 week was 1.95 kg in the combination diuretic group and 0.44 kg in the conventional diuretic group; and therefore, we suspect a difference of at least 1.5 kg/week between the two groups (the standard deviation of the two groups was 1.8 kg). In order to detect a difference of this magnitude that is significant with a 95% confidence interval and a power of 80%, there was a minimum of 24 patients required in each group. Assuming that 20% of the patients would drop out of the study, the required sample size for this trial was, therefore, estimated to be approximately 60 patients (30 patients each in the furosemide and tolvaptan groups).”

Comment 2: It will be more clear if changes in ascites volume after the administration of drugs could be displayed in combination with changes in body weight in Figure 1 and Figure 2 as a direct comparison;

Reply: We have added the following the Figure. (Figure 5):

Comment 3. The occurrence of hyponatremia is an important issue and should be adequately discussed.

Reply: We agree that this point requires, and have added the following text to the Discussion (p. 17, lines 4-12)

“Furthermore, hyponatremia and hypokalemia were not seen in the combination diuretic group. To avoid an electrolyte disturbance, combination diuretic therapy should be evaluated and may resolve hyponatremia with long-term treatment [7]. Hyponatremia, which is characterized by excessive renal retention of water increased release of arginine vasopressin, is associated with increased mortality and numerous complications in cirrhotic patients. A combination therapy, including a vasopressin V2-receptor antagonist, which increases the serum sodium concentration, has the potential to improve outcomes in liver cirrhosis patients with ascites [7].”

Our response for Reviewer 2.

Thank you for your detailed comments that have helped us to improve our manuscript.

Comment 1: The manuscripts title is metaphorical it should be clearer and rephrased.

Reply: We thank the reviewer for this comment. Accordingly, we changed the following the manuscript title.

“Efficacy of combination therapy with natriuretic and aquaretic drugs in cirrhotic ascites patients: A randomized study”

Comment 2: The aim of any study should be reflected in the title. In abstract section aims should be clearly defined and should matches with title. 2. Abstract should be rephrased and brief methodology should be included. 3. Conclusion should be more comprehensive.

Reply: In accordance with the reviewer's comment, we changed the following the abstract.

“AIM

To assess the effects of a combination therapy with natriuretic and aquaretic drugs in cirrhotic ascites patients.

METHODS

A two-center, randomized, open-label, prospective study was conducted. Japanese patients who met the criteria were randomized to trial group and the combination diuretic group (received 7.5 mg of tolvaptan) or the conventional diuretic group (received 40 mg of furosemide) for 7 days in addition to the natriuretic drug which was used prior to enrolment in this study. The primary endpoint was the change in body weight from the baseline. Vital signs, fluid intake, and laboratory and urinary data were assessed to determine the pharmacological effects after administration of aquaretic and natriuretic drugs.

CONCLUSION

Compared to a conventional diuretic therapy with only a natriuretic drug, a combination diuretic therapy with natriuretic and aquaretic drugs is more effective for patients with cirrhotic ascites.”

Comment 3: Introduction: Authors should include more substance about resistant ascites and refractory ascites. Role of Aquaretics should be discussed in case of both resistant and refractory ascites. These additions will make the rationale more appealing.

We agree that this point requires, and have added the following text to the introduction (p. 6, lines 19-26)

“Refractory ascites is composed of diuretic-resistant ascites and diuretic-intractable ascites. Diuretic-resistant cannot be mobilized or the early recurrence because of a lack of response to dietary sodium restriction and conventional diuretics. For the treatment of diuretic-intractable ascites an effective diuretic dosage has not yet been determined because of the development of severe diuretic-related side effects [9]. The strategy of ascites refractory to diuretic therapy has still not been established. ”

Comment 4

Methodology: 1. Authors should define an ineffective diuretic response. The refractory ascites is defined as immobilization of free fluid from peritoneum despite 160 mg or aldactone of 400mg. The refractory ascites is further divided into diuretic resistant or diuretic intractable.

Methodology:2. How ≥ 20 mg furosemide and ≥ 25 mg aldactone for Diuresis explains ineffective diuretic response, as the role of aquaretics is more pronounced in diuretic resistant ascites and dilutional Hyponatremia.

Reply:

We strongly agree the reviewer's concerns on this point. However, our study protocol was according to a diuretics regimen in Japan. This regimen does not recommend to use high-dose diuretics (furosemide of 160 mg or spironolactone of 400m). To avoid acute kidney injury and electrolyte disturbances caused by high-dose diuretics, they propose the combined use of tolvaptan and diuretics for ascitic patients. Therefore, our enrolled patients were a daily dose of ≥ 20 mg furosemide and ≥ 25 mg spironolactone.

The guidelines for liver cirrhosis is listed below,

Reference 13

Fukui H, Saito H, Ueno Y, et al. Evidence-based clinical practice guidelines for liver cirrhosis 2015. *J Gastroenterol* 2016; 51: 638

【CQ: Are vasopressin V2 receptor antagonists effective for management of ascites or water retention in cirrhotic patients?

- A vasopressin V2 receptor antagonist combined with loop diuretics and aldosterone antagonists is recommended for such patients on the basis of its effectiveness on hyponatremia and ascites. (Evidence level A, strength 1)

Comment: Given the central role of vasopressin in limiting renal water excretion in cirrhotic patients, use of vasopressin V2 receptor antagonists is a rational approach for ascitic patients. Among them, tolvaptan (7.5–30 mg/day for 7 days) showed add-on effects to conventional diuretics on ascites in multicenter RCTs for poor responders to the standard diuretic therapy (furosemide at 40 mg/day or greater and spironolactone at 25 mg/day or greater; or furosemide at 20 mg/day or greater and spironolactone at 50 mg/day or greater) [150, 151]. As a dosage of 7.5 mg/day showed the maximum effects with preferable tolerability [151] and a dosage of 3.75 mg/day also exerted significant effects [152], the proper dosage was determined as 3.75–7.5 mg/day. The effects were unrelated to serum albumin levels [150]. Minor increases in serum creatinine levels defined as acute kidney injury by the International Club of Ascites adversely affect survival of cirrhotic patients [153, 154]. To avoid acute kidney injury and electrolyte disturbances caused by high-dose diuretics, we propose the combined use of tolvaptan and diuretics for ascitic patients.】

Furthermore, definition of ineffective diuretic response was that body weight did not loss in spit of administration of intensive diuretic therapy for the 7 days.

To make this point clearer, we have added the following to the Discussion (p. 8, lines 18-21):

“Additionally, the diuretic dosages should not have been changed for at least 7 days prior to initiating the trial, and an ineffective response was one in which body weight was not reduced in spite of administration of intensive diuretic therapy for the 7 days.”

Comment 6:

Methodology: 3. Although authors have given mean CTP score in table but all enrolled cirrhotics should be classified into CTP stages. This severity classification would further clear the role of aquaretics.

Reply: We thank the reviewer for this comment. Accordingly, we added the table.(Table1)

“Child-Pugh stage: A/B/C n 0/16/12 0/15/13 0.7881 ”

Comment 7: 4. Stages of ascites should be determined as it would further help to understand effective role of aquaretics in each stage of ascites.

Reply: We thank the reviewer for this comment. Accordingly, we added the table. (Table1)

“Grade of ascites:mild/moderate/severe n 5/15/8 6/13/9 0.8901 ”

Our response for Reviewer 3.

Thank you for your detailed comments that have helped us to improve our manuscript.

Comment 1: I think the the follow-up period of the study (7 days) is short, and it is not sufficient to evaluate the effects of treatment with tolvaptan for cirrhotic ascites and give the convincing conclusion. Therefore, I suggest that the follow-up period ought to be more than 4 weeks, and the effects should be respectively evaluated in 1,2,3 and 4 weeks.

Reply: We agree that additional information on our report as the reviewer suggested would be valuable. Regrettably, however, we are not able to evaluate in 1,3 weeks. We added the following explanation in the Text (P.14, Lines20-30 and P.15, Lines1-3). And, we are now investigating this point and intend to report it in a later paper (https://upload.umin.ac.jp/cgi-open-bin/ctr_e/ctr_view.cgi?recptno=R000027509).

“Clinical course after treatment for 1 week

After the treatment period, we conducted an observation for 1 month. After the administration of tolvaptan, according to efficacy and tolerability, dose of tolvaptan was changed. Fifteen patients were kept on 7.5 mg, 8 patients were decreased to 3.75 mg; however, for 5 patients the therapy was discontinued because of inefficacy. The average decrease in body weight from baseline was -4.01 ± 3.97 and -4.31 ± 4.07 kg (2 w, 4 w; respectively).

After the administration of furosemide, tolvaptan was administered or the furosemide dose was changed. Twelve patients were treated with tolvaptan. The furosemide doses were decreased for 9 patients. No changes were made in the doses for 5 patients, and only 2 patients received an increased dose of furosemide. Except for the patients who received tolvaptan, the average decrease in body weight from baseline was -3.21 ± 3.15 and -3.61 ± 3.37 kg (2 w, 4 w; respectively).

Comment 2: Patients included in this study had normal renal function, but hepatorenal syndrome may affect the response to diuretics and tolvaptan. The results would likely have been different;

Reply: We appreciate the reviewer's interest in correlation between renal function and response to diuretics. We already reported close correlation between renal function and response to tolvaptan in liver cirrhosis patients with ascites. As the diuretic response to tolvaptan was considered to be dependent on primitive urine in the collecting tubules or on renal blood flow, renal function were useful for the prediction of diuretic response to this drug in liver cirrhosis patients. Especially, BUN and the urine excretion sodium was useful for a prediction of the response to this drug. To make this point clearer, we have added the following to the Discussion (p. 17, lines 20-26).

“Patients enrolled in this study had a relatively good renal function and good sodium excretion; however, if they had presented with mild or severe renal dysfunction, which would have affected the response to diuretics, the results would likely have been markedly different. A previous study reported that, in changes in body weight, according to the patients’ characteristics, are dependent on the level of creatinine or eGFR (estimated glomerular filtration rate) [25].”

Our response for Reviewer 4.

Thank you for your detailed comments that have helped us to improve our manuscript.

Comment 1: The study population is small.

Reply: Sample size was based on previous studies. To make this point clearer, we have added the following to the Discussion (p. 10, lines 18-28):

“Our estimation of clinical effects using change in body weight after a combination diuretic therapy for a week was based on a previous study [11]. The change of body weight after 1 week was 1.95 kg in the combination diuretic group and 0.44 kg in the conventional diuretic group; and therefore, we suspect a difference of at least 1.5 kg/week between the two groups (the standard deviation of the two groups was 1.8 kg). In order to detect a difference of this magnitude that is significant with a 95% confidence interval and a power of 80%, there was a minimum of 24 patients required in each group. Assuming that 20% of the patients would drop out of the study, the required sample size for this trial was, therefore, estimated to be approximately 60 patients (30 patients each in the furosemide and tolvaptan groups).”

Comment 2: The follow up period is short.

Reply: We agree that additional information on our report as the reviewer suggested would be valuable. We added the following explanation in the Text (P.14, Lines20-30 and P.15, Lines1-3).And, we are now investigating this point and intend to report it in a later paper

(https://upload.umin.ac.jp/cgi-open-bin/ctr_e/ctr_view.cgi?recptno=R000027509).

“Clinical course after treatment for 1 week

After the treatment period, we conducted an observation for 1 month. After the administration of tolvaptan, according to efficacy and tolerability, dose of tolvaptan was changed. Fifteen patients were kept on 7.5 mg, 8 patients were decreased to 3.75 mg; however, for 5 patients the therapy was discontinued because of inefficacy. The average decrease in body weight from baseline was -4.01 ± 3.97 and -4.31 ± 4.07 kg (2 w, 4 w; respectively).

After the administration of furosemide, tolvaptan was administered or the furosemide dose was changed. Twelve patients were treated with tolvaptan. The furosemide doses were decreased for 9 patients. No changes were made in the doses for 5 patients, and only 2 patients received an increased dose of furosemide. Except for the patients who received tolvaptan, the average decrease in body weight from baseline was -3.21 ± 3.15 and -3.61 ± 3.37 kg (2 w, 4 w; respectively).

Comment 3: The discussion part is short, it must be longer and would include current studies about the usage of tolvaptan in cirrhotics.

Reply: In accordance with the reviewer's request, we added the manuscript by 3,051 words to a revised total of 3,673 words. Especially, we discussed about the appropriate dosages of aquaretic and natriuretic drugs.

Thank you again for your comments on our manuscript. I trust that the revised manuscript is now suitable for publication in the ***World Journal of Gastroenterology***.

Haruki Uojima, MD
Department of Gastroenterology, Internal Medicine
Kitasato University School of Medicine
1-15-1 Kitasato, Minami-ku, Sagamihara
Kanagawa 252-0375, Japan
Tel: +81-42-778-8111
Fax: +81-42-778-8390
E-mail: kiruha@kitasato-u.ac.jp