Format for ANSWERING REVIEWERS

May 15, 2013

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: ESPS Manuscript No.2979-review.doc).

Title: Hepatic arterial infusion chemotherapy in hepatocellular carcinoma with portal vein tumor thrombosis

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The manuscript has been improved according to the suggestions of reviewers:
1 Format has been updated

2 Revision has been made according to the suggestions of the reviewer

(1) Response to comments of Reviewer 02519881

I congratulate the authors for the attempt of this rather novel approach but I would like to ask how this approach is different from TACE without the embolisation part and did the authors experience any problems with hepatic artery thrombosis. Furthermore, did the authors had experience in patients with less advanced disease?

Response : In the way that chemotherapeutic agents are infused through hepatic artery, transarterial chemoembolization (TACE) without the embolization and hepatic arterial infusion chemotherapy (HAIC) are similar. However, HAIC can give higher dose of chemotherapeutic regimen for longer duration than TACE through the implantable port system. On the other hand, chemotherapeutic agents cannot be selectively infused to tumor in HAIC in contrast to TACE without embolization. Thus, HAIC is more suitable to huge HCCs.
Hepatic arterial thrombosis developed in 4 patients among 50 patients. However, thrombolysis by urokinase was effectively performed, and port removal was needed in only 1 patient. These sentences are added in the page 8.
Although HAIC were used for treating advanced HCC mostly, we often use HAIC in patients with intermediate stage HCC. However, these patients also had massive tumor burden without portal vein tumor thrombosis or refractory HCCs to previous treatment. TACE are used mainly in intermediate stage HCC patients rather than HAIC.

(2)Response to comments of reviewer 02462197

March 30, 2013 World Journal of Gastroenterology ESPS Manuscript NO: 2979 Title: Hepatic arterial

infusion chemotherapy in hepatocellular carcinoma with portal vein tumor thrombosis This is a retrospective monocentre study aimed to evaluate the prognostic factors and anti-tumor effects of hepatic arterial infusion chemotherapy in patients with large hepatocellular cancers and portal vein tumor thrombosis.

GENERAL COMMENTS The results of the present manuscript are interesting, due to the potential alternative role of locoregional chemotherapy vs. use of sorafenib in HCC patients, even in the presence of distant metastases and/or vascular invasion; Despite the present study is not innovative, and several recent studies have been published on the same argument (Miyaki D, et al. J Gastroenterol Hepatol 2012; Baek YH, et al. World J Gastroenterol 2012), it is interesting to underline that the current research is focalized also on patients with distant metastases and/or vascular invasion

SPECIFIC COMMENTS

1. Abstract: it clearly reports the objectives of the study; however, the Authors need to explain some of the abbreviations presented in this part (i.e., HAIC, HCC, PVTT, ECF)

> Response : In response to this comment, we explained the abbreviations, such as HAIC, HCC, PVTT, and ECF. The explanation was provided in 'Material and Methods' due to the limitation of word number in 'Aim'.

2. Material and methods: it is not completely clear how many patients were finally enrolled for the study. Probably, it is better to anticipate the part dedicated to the selection criteria with respect to the sentence: "Fifty of these 68 patients had PVTT, received more than two cycles of HAIC and were enrolled in this study."

> Response : In response to this comment, we changed "Fifty of these 68 patients had PVTT, received more than two cycles of HAIC and were enrolled in this study" to "Among these 68 patients, 50 patients who had PVTT and received more than two cycles of HAIC were enrolled in this study".

3. In some parts in the abstract it is reported that only patients with HCC ≥ 10 cm were enrolled for the study. It is not clear for me why this statement is not reported in the selection criteria: moreover, the 400 cm3 proposed cut-off corresponds to a single lesion of 9 cm of diameter, inferior to the so-cited 10 cm. Finally, in table 1 the inferior range of tumor volume corresponds to a single lesion of less than 4 cm of diameter. Authors need to better clarify this aspect, eventually removing from the abstract the sentences regarding the selection of patients with lesions bigger than 10 cm.

> Response : In response to this comment, we deleted the phrase 'tumors ≥ 10cm'.

4. The meaning of ECF must be explained in the text when it is reported the first time

> Response : We added the explanation for ECF in the first sentence of 'Chemotherapeutic regimen and additional therapy'

5. Construction of multivariate analyses on a population of only 50 patients looks to me not completely correct from the methodological point of view. Elevated risks of colinearity and singularity phenomena are expected in this case. An accurate analysis of goodness of covariates fitting must be performed using specific tests. Authors must underline the limit of the numerosity of the sample size in Discussion.

Response : We added the limitation of small sample size in the discussion. (Manuscript page 11)
We verified the proportional hazard assumption using a log-minus-log survival plot, and evaluated the fit of the model by Cox-Snell residuals. These are added in Statistical analysis part of 'MATERIAL and METHODS' (Manuscript page 6). In addition, we performed statistical analysis in another way due to probable colinearity between post-treatment variables, such as objective response and disease control. Univariate analyses using Cox-proportional hazard regression model were performed. To determine the significant pretreatment factors for survival, multivariate analyses were performed by Cox-proportional hazard regression model. In the multivariate analysis of post-treatment factors for survival, hazard ratios were adjusted for tumor volume≥400cm^3 and pre-treatment PIVKA-II level which were statistically significant pretreatment factors.

6. Moreover, and it the real problem of the paper, it is completely unclear for me in which way disease control and PIVKA reduction are risk factors. However, looking at the results of the multivariate models, hazard ratios (no the odds ratios, you used a Cox regression model!) are > 1. Authors must reevaluate their analyses, eventually selecting no more than 2-3 covariates in each case, and carefully looking at the way in which they doomed their variables and in which way they inserted them in the statistical software

Response : We changed the OR to HR. We described the statistical process in the Statistical analysis part of MATERIAL and METHODS (Manuscript page 6) and response to comment 5. Table 3 and 4 were also changed to show (adjusted) hazard ratio more precisely. In addition, because the hazard ratios were adjusted for tumor volume and pretreatment PIVKA-II level in the multivariate analysis of post-treatment variables, covariate in each case were not more than 3.

(3) Response to comments of reviewer 02444769

This is a case-only clinical trial that evaluated the prognostic factors and anti-tumor effects of HAIC in patients with HCC tumors ≥ 10 cm and PVTT. They concluded that HAIC may be considered as an effective treatment modality for advanced HCC with PVTT in patients with tumors ≥ 10 cm. In general the authors provided new but weak information that might help clinical decision of advanced HCC. But the design of this study is questionable therefore the information authors try to convey to our society might be misleading.

(1) Major points: Major concerns come from the heterogeneity of the patients. Materials & Methods, last paragraph: "During or after the HAIC treatment, additional therapies were performed as necessary, depending on the tumor responses to HAIC, performance status, and hepatic function. Additional treatment included targeted therapy with sorafenib, external radiation therapy, transarterial chemolipiodolization (TACL), systemic chemotherapy, local therapies, such as radiofrequency ablation

(RFA) or percutaneous ethanol injection (PEI), or surgical treatment". And worse, RESULTS, Patients Characteristics, "Twenty-four patients (48%) received previous treatment, and the most common previous treatment was TACL." When you apply so many modalities to the 50 patients, how could you justify the real effect of HAIC? Why a control group was absent (either TACE, radiology, Sorafenib or placebo)? For example, Sorafenib is the standard care of BCLC-C patients. And as claimed by the authors, all patients were Child-A/B thus these patients are amenable to TACE. By doing so, at least we would know the HAIC modality is superior or not. It is possible to achieve these data from the institute.

Response : I agree that heterogeneous treatment modality before and during HAIC can cause bias in this study. As you know, advanced stage HCCs are heterogeneous disease including portal invasion or extrahepatic spread.
The patients of this study were also heterogeneous as you mentioned above. In these heterogeneous patients with non-resectable HCC, multimodality treatment options is needed for a successful treatment (Oncology 2011;81(suppl 1):134–140). Therefore, we treated these patients with combined treatment modality, and multimodality treatment including HAIC led to a significant longer survival in this study.
Since multimodality treatment in this study can cause bias, a prospective study with HAIC is needed in the near future. Especially, a comparative study between HAIC and sorafenib is essential because sorafenib is the standard treatment in BCLC-C patients.
Please consider and understand that this study was retrospectively performed, not prospectively.

We recognize the limitation in this study. Therefore, we added this sentence to the discussion; 'In addition, this study may have inherent bias associated with a small sample size and heterogeneous treatments.' (Manuscript page 11)

Minor points:
(1) Why these patients were not amenable to TACE but good for HAIC?

Response : In this study, the reason that we treated these patients with HAIC instead of TACE is as follows

1. The previous studies of ours reported that high-dose HAIC was more effective in intractable, advanced HCC (Korean J Hepatol 2010;16:355-361, *Cancer chemotherapy and pharmacology* 2010; **65**(2):373-382).
2. Most of the study population had large volume of HCCs involving both hepatic lobes and main portal vein tumor thrombosis. Main portal vein tumor thrombosis is contraindication of TACE (AASLD practice guideline, 2010). We used HAIC in the patients with large volume of HCCs involving PVTT or both hepatic lobes to avoid liver failure after TACE.
3. Those who received previous treatment were the patients who had refractory HCCs to previous treatments, especially TACL. This information is added to 'Material and Methods' (Manuscript page 4).

(2) The target of HAIC is liver mass, why extrahepatic metastasis did not influence survival?

Response : There are some reports that the mortality in advanced HCC is related to intrahepatic tumors and the leading cause of death is intrahepatic tumor progression (Journal of Gastroenterology and Hepatology **27** (2012) 684–689, *World J Gastroenterol* 2007 January 21; 13(3): 414-420). In this study, all patients had advanced intrahepatic HCC with vascular invasion. Since the survival of these patients was influenced by intrahepatic tumor progression, extrahepatic metastasis might not influence the overall survival. This statement was added to discussion (Manuscript page 9)

(4) Response to comments of reviewer 02441335

This is a good article about the treatment modality for advanced HCC with PVTT in patients with tumors ≥ 10 cm by using HAIC, which shows HAIC is an effective treatment modality. I have several questions for the authors:

1. Please mention in the text how many cases were histologically diagnosed?

Response : Two patients were histologically diagnosed.

2. Why the removal of thrombus was not performed in your patients?

Response : Most of the study population had large volume of HCCs involving both hepatic lobes and many patients had extensive portal vein tumor thrombosis. In addition, most of them had cirrhosis. We therefore chose HAIC as the first treatment rather than surgical approach.

3. In Table 1 of baseline patient characteristics, we know the types of portal vein thrombosis(Vp2/Vp3/Vp4) and tumor volume (cm3), but we also hope to see the changes of these parameters after HAIC, in order to evaluate the treatment efficacy.

Response : Unfortunately, we did not check the change of tumor volume after HAIC.

Response of portal vein tumor thrombus (PVTT) was assessed by dynamic CT or dynamic MRI. Regression of PVTT was seen in 13 patients, stable PVTT in 21 patients, and progression in 16 patients.

(5) Response to comments of reviewer 02461636

GENERAL COMMENTS

In the manuscript entitled "**Hepatic arterial infusion chemotherapy in hepatocellular carcinoma with portal vein tumor thrombosis (Prognostic factors of arterial chemotherapy)**", Song et al have evaluated the prognostic factors and anti-tumor effects of HAIC in patients with HCC tumors ≥ 10 cm and PVTT". The manuscript is overall well written and is significant considering the limited treatment options available for HCC.

SPECIFIC COMMENT:

Title: Is appropriate and reflects the intent of the paper

Abstract: Appropriate

Materials: The statistical methods used are appropriate for this study and detailed description appropriate for reproduction by other groups is presented.

Results and Discussion: The sample size for this study is appropriate. The authors have included 16 patients with extrahepatic metastasis at the initiation of HAIC. But all the figures show the cumulative survival of the responders and non-responders. The authors specifically mention in the discussion that *"First, we included patients with extrahepatic metastasis even though HAIC is considered effective only for the treatment of intrahepatic tumors [[7]]. However, extrahepatic metastasis was not independently associated with survival, and the results were as good as those of previous studies despite the inclusion of patients with extrahepatic metastasis".*

Based on the above discussion, the authors must also include in the results some analysis on the extrahepatic metastasis group alone because they claim that the results of survival of this group was as good as that of previous studies. Although this group is not independently associated with survival, since this is the first report of a combination of 5-FU, cisplatin and epirubicin using HAIC, it would be important for the readers to know the independent effect of this regimen on extrahepatic metastasis group.

> Response : We added this information to 'Prognostic factor of survival' in RESULT. (Manuscript page 7 and figure 3)

References: are updated

Tables and figures: Represent the claims of the paper, would be good to include a table or figure on the effect of HAIC on extrahepatic metastasis group.

(6) Response to comments of reviewer 02519060

The authors performed hepatic arterial infusion chemotherapy (HAIC) in 50 patients with large HCC with portal vein tumor thrombus (PVTT), resulting in 6% complete response (CR), 26% partial response (PR), and 44% stable disease (SD), and found that a tumor volume less than 400 cm3 and normal

PIVKA-II were the significant pretreatment prognostic factors. This paper is potentially interesting because HAIC is expected to be an alternative therapy for advanced HCC patients. However, there are critical flaws that preclude recommendation for acceptance. Enrollment criteria are not clearly determined in the study. This will prevent journal's readers to interpret the results properly.

(1) The authors described that patients with HCC >= 10 cm were treated in the abstract, however, they did not mention it in the materials and methods.

Response : We deleted the phrase, 'HCC >= 10 cm'

(2) Approximately half of the patients received previous treatments such as radiation, radiofrequency ablation, or transarterial chemolipiodolization. Therefore, the cohort of this study is quite heterogenous, which makes interpretation of the results difficult. The authors should enroll the patients who had HAIC as an initial treatment, or analyze separately.

Response : Those who received previous treatment were the patients who had refractory HCCs to previous treatments. In addition, these previous treatment had no effect on survival as shown in table 3.

(3) The authors analyzed only the patients who received more than 2 cycles of HAIC. 18 patients are anticipated to have received only 1 cycle of HAIC for some reasons such as disease progression and adverse effects. The authors should analyze all of the patients who received HAIC at least once.

Response : The patients who received 1 cycle of HAIC were seven. Nine patients did not have portal vein tumor thrombus. One patient was Child-Pugh score 8. One patient had portal vein thrombosis, but it did not show enhancement and wash-out in the dynamic MRI.

The treatment of those who received only 1 cycle of HAIC were ceased due to hepatic dysfunction, infection, patient's refusal or follow-up loss, but not ineffective HAIC treatment. Therefore, we enrolled patients who received at least 2 cycles to evaluate the effect of HAIC treatment on the survival rather than other factors.

(4) Did the authors enroll the patients with renal insufficiency, cardiovascular or pulmonary diseases, etc?

Response : No, we did not enroll the patients with serious medical illness. This information is added to MATERIAL AND METHODS (Manuscript page 4); "Exclusion criteria included another concurrent malignancy and other underlying serious medical conditions such as renal or cardiopulmonary insufficiency."

(5) The authors should define PVTT response clearly.

Response : PVTT response was evaluated by dynamic imaging. Response was defined as complete disappearance or at least a 30% decrease in the diameter of PVTT, and non-response was defined as any case that did not qualify for response. This information is added to 'Study assessment' of MATERAL and METHODS (Manuscript page 6)

3 References and typesetting were corrected

Thank you again for publishing our manuscript in the *World Journal of Gastroenterology*.

Sincerely yours,

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