Title: The role of LDH serum levels in predicting global outcome in HCC patients treated with sorafenib: implications for clinical management.

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Version: 2
Date: 28 December 2013

Author's response to reviews: see over
Ancona, 27 December, 2013

Ref: MS: 2003045407112889
Title: The role of LDH serum levels in predicting global outcome in HCC patients treated with sorafenib: implications for clinical management.

Dear Editor,

Please find attached a revised version of the manuscript.
We found the reviewers’ comments very interesting and we therefore modified our paper accordingly.
All changes have been also summarized in an accompanying letter, which contains a point by point reply to the reviewers’ remarks.
We hope that our paper can be now considered for publication in BMC Cancer.

Yours sincerely,

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Reviewer 1

Minor revisions: spell out the word TKI in the abstract

Authors’ Reply: we do agree with reviewer’s comments and therefore modified the paper accordingly. Please see the abstract section.

Discretionary revisions: in discussion (last 2 paragraphs) I would substitute the sentence
Although a prognostic role cannot be ruled out in our series the apparent effect on progression free survival seems to indicate a predictive ability for LDH serum levels during sorafenib therapy. We also demonstrated that LDH serum levels variations (pre- and post-treatment) might correlate with clinical outcome in HCC patients.

In addition to a better prognosis for patients with absolute low LDH level, we demonstrated that a decrease of LDH level during treatment seems to predict a better outcome of HCC patients treated with sorafenib.

Authors’ Reply: we do agree with reviewer’s comments and therefore modified the paper accordingly. Please see the discussion section.

Reviewer 2

The paper “The role of LDH serum levels in predicting global outcome in HCC patients treated with sorafenib: implication for clinical management.” By Faloppi et al. described data regarding the prediction power of LDH (pre-treatment levels and variation during treatment) in HCC patients receiving sorafenib. They concluded that LDH is able to predict clinical PFS and OS of HCC patients receiving sorafenib. The idea of this study is not novel and the results are largely limited by a relatively small (only 78) and heterogeneous group of patients. Furthermore there are several issues needed to be clarified:

1. Basic characteristics of the 78 patients enrolled in this study were not clearly demonstrated and the following questions should be further addressed in the results:
Is this a single center study?

Authors’ Reply: the study in multicentre. Two centres in Italy (Translational Oncology Unit and Gastroenterology Department, AOU "Ospedali Riuniti" – Università Politecnica delle Marche, Ancona; Internal Medicine, Department of Medical Sciences Experimental and Clinical - Università di Udine, Udine) are involved in the study at present. We have add this information in the manuscript. Please see methods section of the manuscript.
How were these patients selected and enrolled for analysis?

Authors’ Reply: From 2008 to 2012 all consecutive patients with advanced HCC or intermediate stage HCC refractory to or unsuitable for locoregional therapies, either histologically proven or diagnosed according to the AASLD guidelines (American Association for the Study of Liver Diseases 2005) and receiving sorafenib were eligible for our analysis. We have explained this information in the manuscript. Please see methods section of the manuscript.

Were there any patient excluded?

Authors’ Reply: from the analysis were excluded only patients without LDH serum levels at the time of treatment.

How was HCC diagnosed and treated? Were there guidelines or consensus of diagnosis and treatment applied to all the patients? Is sorafenib the first-line treatment? If not, what were the treatments prior to sorafenib? Did any patient receive surgical resection?

Authors’ Reply: All patients receiving diagnosis according to the AASLD guidelines (American Association for the Study of Liver Diseases 2005). For all patients sorafenib was the first line systemic treatment. Patients have received locoregional treatment or surgery as part of their treatment history when clinically indicated. We have specified the information about previous treatments in table 1.

For the patients with virus hepatitis, did they receive specific anti-HBV or anti-HCV therapy?

Authors’ Reply: All patients received a gastroenterological evaluation and a specific anti-viral therapy was applied when indicated.

Is there any patient receive PET scan?

Authors’ Reply: no patients underwent PET scan during follow-up.

Is there any patient lost during follow-up?

Authors’ Reply: no patients were lost during follow-up.

2. The authors used ROC curve analysis and found pre-treatment LDH serum level > 407 U/L (should be IU/L?) and LDH elevated one month after sorafenib treatment were significantly
associated with PFS and OS after sorafenib. Since LDH is a well-accepted prognostic marker in many malignant disease, included HCC, and serum level >407 IU/L is actually much higher than the upper normal limit, this cut-of-point may just be a surrogate for higher tumor burden or worse comorbidities, rather than a predictive marker for sorafenib. Furthermore, the cut-off-point (407 IU/L) used in this study was calculated by ROC analysis from their own study population without further external validation. Would this result be able to apply to other study population? (the author had published another cut-of-point as 450 IU/L in prediction outcome of HCC patients post TACE, so which value is of biological significance? The author need to clarify this or at least discuss this)

In addition, the author chose the one-month-dynamic change of LDH as another important predictive marker of sorafenib treatment. The LDH change may reflect the tumor burden change of patients and is of certain clinical significance. However the change should be carefully assessed since the post-treatment LDH might be influenced by side effect of sorafenib. The author should present the percentage of side effect between these two groups (LDH increase and decrease) and also explain the rational of using “one-month” as the period of investigation

Authors’ Reply: Thank you for the comment.

We confirm that U/L is correct in our analysis.

In our laboratory upper normal limit for LDH serum levels is 450 U/L (as it is now indicated in the text, please see Patients and Methods section of the paper). Our previous report was a mono institutional analysis, however in the present analysis we have decided to use the cut-off 407 U/L, calculated by ROC analysis, to eliminate a potential bias deriving from the inclusion of patients from different Centres.

Regarding LDH we have decided to use a one-month-dynamic change in order to investigate whether LDH might be used as an early predictor of sorafenib failure. We have added in the manuscript the toxicities profiles in the patients groups (please see table 2) and we have explained in the manuscript the rational of using “one-month” as the period of investigation. Please see methods section of the manuscript.

3. Because LDH has many isoforms and the serum level of total form could be easily influenced by many conditions other than HCC (like infection, inflammation, hemalysis, and etc.), using a the level of a more specific isoform may be more informative. Additionally, the level of LDH should be adjusted with other important factors like age, sex, stage, etiologies of HCC, liver reserve (like ALT, ALB), CRP, viral load… (using log-rank test only could not exclude the impact of other confounding factors).
Authors’ Reply: Thank you for the comment. The study of LDH isoforms, in particular LDH-5, is very interesting, but the assay of LDH isoforms is not routinely performed in the Institutions involved in the analysis. Furthermore due to the retrospective nature of our study this information is not available. We have revised the statistics of our study. All variables requested were included in the analysis. Please see methods and results sections of the manuscript and table 1.