ANSWER TO REVIEWERS

October 26, 2013

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 5587-review1.doc).

Title: A randomized trial of iron depletion in patients with NAFLD and Hyperferritinemia

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Name of Journal: *World Journal of Gastroenterology*

ESPS Manuscript NO: 5587

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) **The study examines the treatment of NAFLD patients with phlebotomy. This treatment modality has been examined in a number of other studies with variable results. There are several concerns with the present study that compromise its significance. Specific comments 1. A NAS as low as 1 does not qualify as "severe NAFLD" as the authors state in the Methods. 2. Because there is a high rate of spontaneous improvement in NAS of 1 point, the accepted endpoint for clinical trials is an improvement in NAS of 2 points with no worsening of fibrosis. The data should be reinterpreted by that criteria. 3. Under the study design in this paper a patient with a NAS of 1 based on mild steatosis alone who had resolution of the steatosis would be a treatment success. 4. The study is underpowered with only 19 patients completing the study. There was no statistical difference in the intention to treat analysis.** We thank the Reviewer for the insightful suggestions. 1. Inclusion criteria for our study were the presence of NAFLD with NAS>1 (not > or = 1 as uncorrectly stated in the methods of the previous manuscript version, see the online study protocol of the study; we apologize for the mistake and we have now correcte this information in the Methods section) AND hyperferritenima DESPITE at least 6 months of lifestyle changes, a risk factor for liver disease severity, that we arbitrarily defined as "severe NAFLD associated with hyperferritinemia". 2. The Reviewer refer to accepted criteria of liver damage improvement in clinical studies in patients with NASH. We would like to point out that those criteria were not accepted at the time the protocol of the present study was approved, and that we did not enrol patients with NASH, but patients with a different factor possibly associated with liver damage progression in NAFLD (hyper-ferritinemia with mild iron accumulation). See the Discussion section for the literature supporting the choice of including patients without definite NASH and possible mechanisms associated with iron related liver damage that are not reflected in the NAS score. Given that enrolment was based on the approved protocol, re-evaluation of the results according to new criteria developed for a clinically different subset of patients with a different spectrum of severity liver disease at baseline would be meaningless. 3. As specified above, NAS>1 was a required criterium for enrolment, but a one point decrease of NAS due to improvement in either steatosis, or necroinflammation, or ballooning without worsening of fibrosis has been considered as a treatment success. Despite continuos support for lifestyle changes, the spontaneous improvement rate in histological liver damage and liver enzymes was significantly lower in the control group. 4. We thoroughly discussed the strong limitations of our study in the discussion section, and the conclusions have accordingly taken these limitations into account. However, several secondary endpoints were met

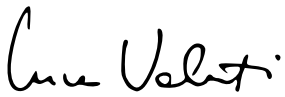
suggesting that this therapeutic approach may be worth further testing in selected group of patients. We now specified even more clearly these limitations in the discussion section.

(2) **minor revision need.** We thank the Reviewer for the positive comment on the manuscript.

(3) **please correct: Page 10, lver; Effect of treatment of liver enzymes Page 13, rate of improvement of liver damage improvement Page 6, thyoroid Please show in a diagram individual steatosis grades, necroinflammation and hepatocellular ballooning of the 19 patients before and after finishing the study. The authors have to clearly indicate why this study was performed or indicate differences of this and their earlier study because similar findings have already been published by this group (Am J Gastroenterol. 2007 Jun;102(6):1251-8.).** We thank the Reviewer for the useful suggestions and corrections that have been incorporated into the manuscript. In particular, we have stressed the novelty of the present study at the beginning of the Discussion section (randomized clinical trial), and we have added a figure (new figure 4) presenting the variations in histological indices of liver damage in individual patients subdivided according to treatment arm.

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

Sincerely yours,



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