October 6, 2013

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 4716-review.doc).

Title: The prognostic significance of nonalcoholic fatty liver disease in patients with acute ischemic stroke

Authors: Konstantinos Tziomalos, Vasilios Giampatzis, Stella D. Bouziana, Marianna Spanou, Maria Papadopoulou, Athinodoros Pavlidis, Stavroula Kostaki, Andreas Bozikas, Christos Savopoulos, Apostolos I Hatzitolios

Name of Journal: *World Journal of Hepatology*

ESPS Manuscript NO: 4716

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 reviewers.

Reviewer 02518353
This is an interesting article; even though the results are negative because NAFLD is addressed in acute stroke patients.
We thank this Reviewer for these positive comments.

There are some points that may improve the manuscript.
1. Triglyceride level is a Tg?
Yes, and we define this abbreviation in the Methods (page 7, first paragraph).

2. How many patients who did not drink alcohol at all in the NAFLD group? If re-analyze with this definition, any differences? My understanding is that NAFLD should be diagnosed in patients without alcohol consumption at all.
We thank this Reviewer for this important comment. We added in the Results (page 10, first paragraph) "Among the 32 patients with NAFLD, 24 did not drink alcohol at all. When these 24 patients were compared with the rest of the study population (n = 391), similar results were obtained."

3. Even though there are no significant factors in both outcome analyses, multiple logistic analysis should be done with those factors with p value less than .20 by descriptive analysis. You may find some significant factors.
We added in the Statistical Analysis (page 8, first paragraph) "Multiple logistic regression analysis was performed to identify independent predictors of dependency at discharge and of in-hospital mortality including factors with $p < 0.20$ by descriptive analysis." We also added in the Results (page 9, last paragraph) "In multiple logistic regression analysis, independent predictors of dependency at discharge were age (risk ratio (RR) 1.16, 95% confidence interval (CI) 1.05-1.28, $p < 0.005$), history of stroke (RR 3.66, 95% CI 1.25-10.71, $p < 0.05$) and the NIHSS score at admission (RR 1.61, 95% CI 1.34-1.92, $p < 0.001$). Independent predictors of in-hospital mortality were diastolic blood pressure at admission (RR 1.06, 95% CI 1.01-1.11, $p < 0.05$) and the NIHSS score at admission (RR 1.17, 95% CI 1.10-1.23, $p < 0.001$)."

Reviewer 02456047

1. The rationale of this study should be strengthened in the introduction. The authors should include a paper from Ying et al. (2011) to argue why this present study is needed. Reference: Ying I, Saposnik G, Vermeulen MJ, Leung A, Ray JG. Nonalcoholic fatty liver disease and acute ischemic stroke. Epidemiology. 2011 Jan;22(1):129-30. doi: 10.1097/EDE.0b013e3181feb50a.

We now cite the paper by Ying in the Introduction were we mention "A recent case-control study in 103 patients with ischemic stroke and 200 controls also showed that elevated aminotransferase levels is associated with increased risk for ischemic stroke. However, there is a paucity of data regarding the association between NAFLD and the severity and outcome of acute ischemic stroke. Accordingly, the aim of the present study was to determine the prevalence of NAFLD in patients admitted with acute ischemic stroke and to evaluate the association of NAFLD with stroke severity and in-hospital outcome." Notably, the report by Ying et al did not evaluate the association between elevated transaminase levels and stroke severity or outcome as was done in our study.

2. The authors used ALT values to determine the group of NAFLD. However, Mofrad et al. (2003) found that many individuals with the entire histological spectrum of NAFLD have normal ALT values. Also, it has been suggested that the normal limits for ALT values should be revised and be lowered. So, first, it is unclear to what extent the upper limit of ALT values was used in this present study. Second, is it really appropriate to use ALT values? Any histological examinations were used to more accurately determine the group of NAFLD? Such as ultrasonography, CT or MRI? Reference: Mofrad P, Contos MJ, Haque M, Sargeant C, Fisher RA, Luketic VA, Sterling RK, Shiffman ML, Stravitz RT, Sanyal AJ. Clinical and histologic spectrum of nonalcoholic fatty liver disease associated with normal ALT values. Hepatology. 2003 Jun; 37(6):1286-92.

We thank this Reviewer for these important comments. We mention in the Methods (page 7, second paragraph) "Nonalcoholic fatty liver disease was defined as serum ALT and/or AST levels above the upper limit of normal in the absence of other causes of elevated aminotransferases levels (chronic hepatitis B or C, drug toxicity, increased alcohol consumption (> 21 and > 14 drinks per week in men and women, respectively), cholestatic diseases or rhabdomyolysis)". We also mention in the Discussion (page 10, third paragraph) "the diagnosis of NAFLD in the general population is primarily based on identification of hepatic steatosis with imaging. In contrast, in the present study we defined NAFLD as the presence of elevated aminotransferases levels in the absence of other causes of chronic liver disease. However, it has been reported that less than one third of patients with NAFLD have elevated aminotransferases levels. Therefore, the different definition of NAFLD might have contributed to the lower prevalence of NAFLD in our study." We now cite the paper by Mofrad et al. at this point. Unfortunately, we did not perform any histological examinations to more accurately determine the group of NAFLD. It would be very difficult to perform liver biopsy in a large population of patients with acute stroke (n = 415) who also universally receive either antiplatelet or anticoagulant agents. Regarding imaging studies, we added in the Methods (page 7, second paragraph) "Liver ultrasonography was performed in all patients with elevated aminotransferases levels." and the Results (page 8, second paragraph) "Ultrasonography showed increased liver echogenicity in all patients with NAFLD." Again, it would be very difficult and expensive to perform CT or MRI in 415 patients with acute stroke.

3. The sample size of the NAFLD group is very small and it is about 10% of the non-NAFLD group. Is it really meaningful to compare these two groups from the statistical standpoints? Can the authors expand their sample size for the NAFLD group?

We repeatedly emphasize the limitation of the sample size in the Discussion were we mention "Our results suggest that NAFLD might not be independently associated

with greater stroke severity but larger studies are needed to resolve this clinically important and controversial issue." and "There are no studies that evaluated the association between NAFLD and functional outcome in patients with acute ischemic stroke. Our findings suggest that this relationship might be week or absent but this remains to be confirmed or rejected in larger studies." and "In the present study, mortality rates were higher in patients with NAFLD than in those without but this difference did not reach significance. It is possible that the small number of patients with NAFLD limited the statistical power of our study resulting in a type II statistical error." We also mention in the Conclusions "However, given the small number of patients with NAFLD in the present report and the lack of other studies evaluating this association, larger studies are needed to further evaluate the predictive value of NAFLD in this high-risk population." Regarding the expansion of the NAFLD population, we mention in the Discussion "Apart from aminotransferases, serum γ-GT levels are also frequently elevated in patients with NAFLD. However, when NAFLD was defined as the presence of elevated aminotransferases and/or γ-GT in the present study, again there was no association between NAFLD and stroke severity or outcome (data not shown)." According to this definition, 71 patients had NAFLD compared with 32 patients diagnosed with NAFLD according to the more restrictive definition based only on elevated aminotransferases.
However, despite the small number of patients with NAFLD in our study, this is the first report that evaluates the association between NAFLD and stroke severity and outcome. We therefore believe that despite its limitations, our study adds new knowledge on the important topic of the relation between NAFLD and cardiovascular risk.

4. Were the authors able to use a quantitative index to present the severity of the NAFLD? Then conduct correlation or prediction between this index with the scores of the outcome measures?
Since we did not perform liver biopsy in our patients, we could not use a reliable quantitative index to evaluate the severity of NAFLD. However, given the small number of patients with NAFLD in our study, it is unlikely that the correlation between outcome and the severity of liver steatosis, necroinflammation or fibrosis would have been significant.

Reviewer 00069467
There are still some concerns need to be addressed prior to publication, which are listed below.
(1) There is no mention that whether or not the present study was approved by the local medical ethic commission in materials and methods section;
We added in the Materials and methods section (page 7, last paragraph) "The study was approved by the Ethics Committee of the Medical School of the Aristotle University of Thessaloniki."

(2) The novelty might be discounted by the earlier report by Ying I et al in the form of letter to the editor (Ying I et al, Epidemiology. 2011; 22(1):129-30). Suggest reference it where appropriate;
The report by Ying et al is a case-control study in 103 patients with ischemic stroke and 200 controls that showed that elevated transaminase levels is associated with increased risk for ischemic stroke. However, it did not evaluate the association between elevated transaminase levels and stroke severity or outcome as was done in our study. However, we now cite this study in the Introduction were we mention "A recent case-control study in 103 patients with ischemic stroke and 200 controls also showed that elevated transaminase levels is associated with increased risk for ischemic stroke."

(3) The conclusion arrived by the authors that "NAFLD is not associated with more severe stroke" is at best suggestive rather than conclusive. It would be more careful to replace the word "is" with "may/might be" , especially given the different criteria of NAFLD adopted could lead to different statistical results;
We thank this Reviewer for this comment. We replaced the word "is" with "might be".

(4) The findings of article don't seem to show what the title suggests. Of note, "prognosis" and "outcome" are two different words with distinction meaning which shouldn't be confused;
We changed the title to "The association between nonalcoholic fatty liver disease and acute ischemic stroke severity and outcome"

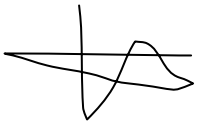
(5) More comments should be given revolving around the connection between fatty liver disease and stroke/cardiovascular disease, instead of separately discussing the findings concerning the two diseases;
We have expanded the discussion of the connection between fatty liver and stroke/cardiovascular disease.

(6) Thus, some references associated with fatty liver disease and stroke/cardiovascular disease had better be included and inserted in the discussion part: Ying I, Saposnik G, Vermeulen MJ, et al,. Nonalcoholic fatty liver disease and acute ischemic stroke. Epidemiology. 2011;22(1):129-30. Targher G, Bertolini L, Poli F, et al. Nonalcoholic fatty liver disease and risk of future cardiovascular events among type 2 diabetic patients. Eur Rev Med Pharmacol Sci. 2005; 9: 269-271. Sookian S, Pirola CJ. Non-alcoholic fatty liver disease is strongly associated with caroid atherosclerosis: a systemic review. J Hepatol. 2008; 49: 600-607. Hamaguchi M, Kojima T, Takeda N, et al. Nonalcoholic fatty liver disease is a novel predictor of cardiovascular disease. World J Gastroenterol. 2007; 13: 1579-1584.
We now include all these important papers in the Discussion.

3 References and typesetting were corrected.

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

Sincerely yours,



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