Format for ANSWERING REVIEWERS

March 17, 2015

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: ESPS Manuscript NO 16353-edited.doc).

Title: Controversy in the diagnosis of pediatric non-alcoholic fatty liver disease

Authors: Pierluigi Marzuillo, Anna Grandone, Laura Perrone, Emanuele Miraglia del Giudice.

Name of Journal: *World Journal of Gastroenterology*

ESPS Manuscript NO: 16353

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

(1) We performed a written English revision

(2) We modified the manuscript title

(3) We added data of NASH prevalence in childhood and discussed the potential diagnostic value of non-invasive methods against this background

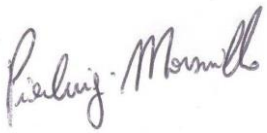
(4) We discussed CAP and ARFI methods in our manuscript

(5) We changed the order of two subtitles "What are the alternatives to a liver biopsy?" and "When to perform a liver biopsy?".

(6)We detailed all the manuscript improvements in the next pages

In the next page there are the detailed answers to reviewers.
We hope that our manuscript will be of interest for the readers of the *World Journal of Hepatology*.

Sincerely yours,



Department of Woman, Child and General and Specialized Surgery
Seconda Univesità degli Studi di Napoli
Via L. De Crecchio n° 2 80138, Napoli, Italy
Fax: 00390815665427
e-mail: pierluigi.marzuillo@gmail.com

We thank the reviewers for taking the time to read and comment on our manuscript. All the changes have been highlighted in the text.

Reviewer 02939985

Comments: Manuscript entitled Controversy in pediatric Non-Alcoholic Fatty Liver Disease diagnosis is a comprehensive, well prepared review of the methods used in clinical practice for diagnosis of the liver steatosis and fibrosis in children. Authors in a clear way present details of the present diagnostic standard and future possibilities. The reference list contains the most important original and review papers in this field. Minor points: Page 4. The sentence: However with the rising prevalence of NAFLD, the proportion of children with both an underlying primary liver disease (e.g.; autoimmune liver disease or Wilson disease) and additional NAFLD increases, and becomes essential not to miss a treatable condition[9] is not clear. I suggest to correct it. Page 18. Reference No 55 should be corrected.

Answer: Thank you, we corrected the indicated sentence (see lines 95-98 of the new version of the manuscript). We also corrected the reference 55.

Reviewer 02939926

Comments: Thank you for the opportunity to review this editorial, which summarizes current methods for non-invasive characterization of NAFLD and their (potential) role in the pediatric setting. Although the major aspects of current methods are mentioned in the article, a revision of the manuscript should consider the following points:

Major aspects:

1) NAFLD in childhood: There is general consent that fatty liver can be considered as part of the metabolic syndrome which reduces overall prognosis. However, the progression from simple steatosis to advanced fibrosis, cirrhosis and further endpoints is a long term phenomenon and liver related events due to NAFLD have a low incidence in children. This is important because prevalence determines the predictive value of (non-invasive) diagnostic methods. Please add data of NASH fibrosis prevalence and incidence of NAFLD related events in childhood and discuss the potential diagnostic value of non-invasive methods against this background.

Answer: thank you for your important observation, we fully agree with you. We added your comment in the text, we added data of NASH prevalence in childhood and discussed the potential diagnostic value of non-invasive methods against this background (see lines 83-91 of the new version of the manuscript).

2) Beside life style modification, treatment options for NAFLD are limited, especially in the pediatric setting. What could be the role of NAFLD screening programs for children by means of non-invasive methods, if the major risk factor (obesity) is easy to diagnose?

Answer: the role of NAFLD screening programs could be to underline the importance of weight loss and then to give more motivations to lose weight to obese children and adolescents. Moreover, these patients could undergo to a follow up program to evaluate the potential NAFLD progression in the following years.

Further aspects:

1) Elastography: a) I suggest using the term "transient elastography" instead of the brand name "Fibroscan".

Answer: we agree with you, see line 195 of the new version of the manuscript.

b) The cited literature on Fibroscan in the pediatric setting is outdated. Meanwhile, the manufacturer provides a small probe (S probe) dedicated to smaller children (e.g. Engelmann G et al. Eur J Pediatr. 2012 Feb;171(2):353-60. doi: 10.1007/s00431-011-1558-7). This probe improves feasibility and allows examination of children of younger age.

Answer: thank you for your important suggestion. We added the reference you suggested us (see line 199-200 of the new version of the manuscript)

c) The controlled attenuation parameter CAP), a software module available for the Fibroscan probes M and XL, allows quantification of hepatic fat content along with assessment of liver stiffness. This is the first non-invasive method for hepatic fat quantification which can potentially be applied in larger cohorts and may be used in screening programs (de Ledinghen et al. J Hepatol. 2014 May;60(5):1026-31. doi: 10.1016/j.jhep.2013.12.018.). Data from adult cohorts are promising and therefore, this technology should be discussed in the manuscript: CAP merrits evaluation in pediatric cohorts.

Answer: thank you for your comment. As suggested, we discussed CAP in our manuscript (see lines 201-206 of the new version of the manuscript).

d) Alternative elastography methods (e.g. Acoustic Radiation Force Impulse Imaging, ARFI) are available, have already been evaluated in the pediatric setting and should therefore be mentioned in the manuscript (e.g. Lee MJ et al. Eur J Radiol. 2013 Jun;82(6):e290-4. doi: 10.1016/j.ejrad.2013.01.018.)

Answer: thank you for your suggestion, we discussed this method in the text (see lines 211-214 of the new version of the manuscript).

2) MR techniques: a) Please discuss costs and availability of these techniques. What might be their role in screening programs?

Answer: as suggested, we discussed costs, availability and role in the screening programs of MR techniques (see lines 183-187 of the new version of the manuscript).

b) When comparing MR techniques with liver biopsy, one need to keep in mind that histology gives a fraction of hepatocytes involved rather than fraction of tissue volume occupied by fat (MR). These different types of units of measure cannot be directly compared ... We currently do not know which method better predicts the outcome of NAFLD.

Answer: thank you for your comment; we added this consideration in the new version of the manuscript (see lines 181-183).

3) Computed tomography: This technique is associated with significant irradiation doses with unknown long term risks, especially when used in children. Regarding the rather benign aspect of pediatric NAFLD, there is no role for CT scans in the diagnosis of this disease. CT scans should be reserved for pediatric emergencies and malignancies.

Answer: we fully agree with you. We added this information at lines 173 and 174 of the new version of the manuscript.

4) Laboratory based approaches: a) Please discuss the role of age-specific upper limits of normal, e.g. for ALT, and their role for non-invasive NAFLD markers.

Answer: as suggested, we discussed the role of age-specific ALT upper limit and their role as non-invasive NAFLD markers (see lines 140-143 of the new version of the manuscript)

b) Is there a potential role for ferritin / iron in the diagnosis of NAFLD severity (e.g. Kowdley KV et al. Hepatology. 2012 Jan;55(1):77-85. doi: 10.1002/hep.24706)

Answer: thank you for your suggestion. We added the potential role of ferritin in the new version of the manuscript at lines 144-146.

Formal aspects: 1) Table one, elastography: "the probe size IN not appropriate" – please correct

Answer: thank you, we corrected that mistake and moreover modified Fibroscan in transient elastography (see new version of table 1).

2) check typesetting of reference 55

Answers: thank you. We corrected the typesetting of reference 55

Reviewer 02508010

Comments: The manuscript entitled "Controversy in pediatric non-alcoholic fatty liver disease diagnosis" reviewed a hot topic for diagnosing controversy of pediatric NAFLD. For the alternatives methods for diagnosis of pediatric NAFLD, ultrasound taking the advantages of noninvasive, low cost, and feasibility is suggested a screening tool in high suspicious population (such in obesity). Although many limits (as descriptions in the text Page 6, lines 2-6) exist, authors and NSPGHAN consensus also suggest ultrasound combined with ALT are reasonable screening tool in the high risk population (such as obesity). This article is a well-written, comprehensive comparison and discussion for the dilemma of diagnosis of pediatric NAFLD. However, some suggestions are list below. The comments to authors:

1. The title may change to "Controversy in the diagnosis of pediatric non-alcoholic fatty

liver disease".

Answer: Thank you. As suggested we modified the title of the manuscript (see line 4 of the new version of the manuscript)

2. I suggest to change the order of two subtitles "What are the alternatives to a liver biopsy?" and "When to perform a liver biopsy?". It is reasonable to discuss liver biopsy, a gold standard for NAFLD diagnosis, first then talk about alternatives.

Answer: We agree with you. As suggested we changed the order of two subtitles.

3. Reference 48. The journal Obesity needs Italic.

Answer: We modified this reference, as indicated.

4. Table 1. ALT seric levels dosage change to ALT serum levels dosage.

Answer: We performed the change you suggested (see Table 1 of new version of the manuscript)