April 3, 2014

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 9722-review.doc).

Title: Non-alcoholic fatty liver disease: what the clinician needs to know

Author: Mariana Verdelho Machado, Helena Cortez-Pinto

Name of Journal: *World Journal of Gastroenterology*

ESPS Manuscript NO: 9722

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

1) References and typesetting were corrected

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

Reviewer 1

This is an excellent review as usually with this team.
We thank the reviewer for the kind words and for the opportunity to improve the manuscript.
Few comments:

1. **Please define precisely PAMPS –** the following explanation was added: "that recognize molecular patterns associated with microbial pathogens or cellular stress"
2. **Please add the proportions of the various sources of FFA in figure 1. –** it was added
3. **Correct some typos:**
   a. **p15, line 11: rennin instead of renin –** corrected
   b. **p16 line 3 : promoter instead of promoters –** corrected
   c. **p18 : Bedossi instead of Bedossa –** corrected
4. **In the management chapter and in table 1, few comments should be added by the authors concerning the screening of HCC in patients with NASH (of course in those with cirrhosis, but also in those without cirrhosis) even if no clear EBM data are available at the moment. Similarly, the usual screening of breast, colo-rectal, prostate cancer etc, according to the specific recommandations should be well followed in these patients due to their relative high risk. This should be added in the management chapter.**
   We thank the reviewer for this very important comment.
   In the chapter of treatment the following paragraph was included: "Screening for hepatocellular carcinoma should be offered to all patients with liver cirrhosis. In patients

with NASH and even simple steatosis with no fibrosis, though there are several reports on the literature of liver cancer, there is not sufficient evidence to recommend a screening program. Though a cost-efficacy analysis has not been done in patients with NAFLD without fibrosis, the incidence of liver cancer in those patients is so low that would not warrant its application. Also, because patients with NAFLD are at increased risk of malignancies, they should be carefully monitored in the regular screening programs for colorectal, prostate, breast and cervical cancer."

In the table it was added the following:

Screening for cancer
Screening for hepatocellular carcinoma every 6 months in cirrhotic patients
Screening program (colorectal, breast, prostate and cervical cancer) as general population

Reviewer 2

The review of Helena Cortez-Pinto "NON-ALCOHOLIC FATTY LIVER DISEASE: WHAT THE CLINICIAN NEEDS TO KNOW" is nice written work, which summarize the modern aspects of the NAFLD development, pathogenesis, epidemiology and natural history. Also authors focus on new specific ways of treatment and non-invasive diagnostic markers for NAFLD depending of it stage which will be helpful for clinicians to make right decisions in NAFLD management. But after before publication must correct some typos.

We thank the reviewer for the kind words and for the opportunity to improve the manuscript. The text was revised and some typos corrected.

Reviewer 3

It is an interesting topic mentioned in this manuscript, presenting the update of NAFLD through epidemiology, pathogenesis, genetics, diagnosis and management views, the manuscript summarized the recent literature inclusively, and organized the materials logically, this review is suitable for people working on this area to read.

We thank the reviewer for the kind words and for the opportunity to improve the manuscript.

1. Basic backgrounds of NAFLD are introduced in detail and indicated in Figs, available managements are demonstrated in Tables, these are good for reader(s). But the dysfunction of lipids induced in pathological state was not conducted.

We completely agree that the role of lipids in the progression of liver injury is a very important topic, and that is why we have the phrase, in page 9:

"Lipid accumulation in the liver is linked with endoplasmic reticulum (ER) stress, oxidative stress/mitochondria stress, and impaired autophagy, resulting in the condition known as lipotoxicity [31]. In some patients, lipotoxicity leads to cell damage and cell death, which induces an inflammatory and wound healing response that can drive fibrogenesis. Why in some people lipid accumulation in the

liver is inert and in others is toxic to cells is still not fully understood. It may be related to the type of fat itself, since triglycerides *per se* do not seem toxic [35], whereas FA, mainly saturated FA [49, 50] as well as cholesterol [51] and its metabolites do seem highly toxic. The higher toxicity of saturated FA as compared to polyunsaturated FA may be in part due to a limited capacity of hepatocytes to use them to produce triglycerides [52]. Also, individual differences in lipid metabolism (e.g. in the enzymes that desaturate FA [50]) and susceptibility for cell damage, may promote NASH development."

2. The pathogenesis part is quite comprehensive, but several issues need to be clarified, such as M1/M2 polymerization, it's confusing as where it is happened that related, liver or adipose tissue?
We thank and agree with the reviewer, it was not clear. As such, we changed the text as following: "In the early phases of NAFLD, the classic activation of macrophages, M1, in the liver, may promote more inflammation and IR, as well as steatosis. However, an alternative activation of macrophages, M2, which is anti-inflammatory and insulin-sensitizer, but also profibrogenic seems to play a major role. In fact, in several mouse models, ob/ob, high fat-diet and lipoatrophic diabetic A-ZIP transgenic mice with fatty liver, an M2 response, in the adipose tissue and in the liver, protects from glucose intolerance and IR, as well as hepatic steatosis [83-85]"

3. Some of the spellings need to be rechecked, such as NAFLD not NAFDL.
We thank the reviewer for this comment, spellings were corrected.

Reviewer 4

It's a highly interesting review which comprises all the main concern about NAFLD (epidemiology, genetics, pathogenesis, diagnosis and management). It's very good written. Although, there are some typos to fix (NAFDL instead of NAFLD in p 22, rennin instead of renin in p15).Also, in the pathogenesis section, the M1/M2 polarization must be further clarified and a brief commment about T cells involvement (th1 profile) must be introduced.
We thank the reviewer for the kind words and for the opportunity to improve the manuscript.
Typos were corrected.
M1/M2 polarization was clarified with the following changes: "In the early phases of NAFLD, the classic activation of macrophages, M1, in the liver, may promote more inflammation and IR, as well as steatosis. However, an alternative activation of macrophages, M2, which is anti-inflammatory and insulin-sensitizer, but also profibrogenic seems to play a major role. In fact, in several mouse models, ob/ob, high fat-diet and lipoatrophic diabetic A-ZIP transgenic mice with fatty liver, an M2 response, in the adipose tissue and in the liver, protects from glucose intolerance and IR, as well as hepatic steatosis [83-85]"
Regarding T cells, we added the following paragraph: "Also, adaptive immune system seems to have a role, having been described a Th1-polarization in the liver and peripheral blood of patients with NASH."

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

Sincerely yours,

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