To the Editor

World Journal of Gastroenteroly

Revised manuscript NO: 35349 **:** ***NONALCOHOLIC FATTY LIVER DISEASE: EVOLVING PARADIGMS . Invited manuscript*** **number ID: 00004847**

Dear Editor

I am submitting the revised manuscript entitled: ***NONALCOHOLIC FATTY LIVER DISEASE: EVOLVING PARADIGMS, by A Lonardo et al. for publication in WJG.***

Point-by-point replay to the comments and suggestions of referees are reported below. All the changes in the manuscript are in red.

Thank you very much to the Editor and to all referees.

Best regards

Prof. Luigi E. Adinolfi

POINT-BY-POINT RESPONSE.

Reviewer #1

The theme presented in the manuscript is relevant in scientific community, extremely interesting and current, and the authors described relevant aspects with fluidity. Some minor revisions should be making.

We wish to thank Rev. 1 for his/her kind comments. We have carefully revised the manuscript for minor revisions, notably including typos, grammatical and spelling errors.

Reviewer #2

This manuscript by Lonardo et al. is a comprehensive review on Nonalcoholic Fatty Liver Disease, thoroughly exploring all the issues concerning this emerging global health problem. The manuscript is well written and conceived.

We wish to thank Rev. 2 for his/her kind comments.

I only have few comments:

- the acronyms should be written in extenso when the authors mention them for the first time

We thank Rev 2 for his/her comment. We have carefully revised the acronyms throughout the manuscript.

- page 6, last paragraph: I suggest to better point out this topic on nuclear receptors to their role in the pathogenesis of NAFLD

We thank Rev 2 for his/her comment. As requested, we have emphasized the role of nuclear receptors in the pathogenesis of NAFLD by adding the following sentence to the manuscript: “Owing to the development of specific targeted drugs, peroxisome proliferator-activated receptors (PPARs), farnesoid X receptor (FXR) and liver X receptors (LXRs), now deserve full attention. The

PPARs superfamily includes PPAR-α, PPAR-β/δ and PPAR-γ. PPAR-α, mainly expressed in liver and muscle, modulates the rates of fatty acids catabolism, lipogenesis and ketone body synthesis by acting as a nutrient sensor. PPAR-γ, abundantly expressed in the adipose tissue, promotes adipocyte differentiation and storage of triglycerides, and regulates glucose homeostasis. PPAR-β/δ, universally expressed in all organ tissues, regulates glucose and lipoprotein metabolism and exerts an anti-inflammatory role. FXR, mainly expressed in the liver and gut, acts as a sensor of bile acids and mediates the signaling effects exerted by bile acid on glucose and lipid metabolism. LXRs serve as lipid sensors in the liver and participate in regulating the metabolism of cholesterol and fatty acids. The role of nuclear receptors in NAFLD pathophysiology has beencomprehensively reviewed elsewhere (15)."

- page 10, second paragraph, line 5: I suggest to better describe the SAF score, because the authors' comments are not easily understandable. A table may be added to compare the Kleiner's classification and the FLIP algorithm

We thank Rev 2 for his/hersuggestion. As we were requested to do, we have now extended the paragraph on the SAF score by adding the following sentence "The SAF score separately assesses steatosis, activity and fibrosis. Activity, which ranges from 0 to 4, is derived from the combination of the semi-quantitative values of hepatocellular ballooning (0-2) and lobular inflammation (0-2). NASH according to the FLIP algorithm is diagnosed when steatosis, ballooning and lobular inflammation are all scored at least 1." Moreover, we have added Table 1, which shows the main features ofNAS and SAF scores and highlights the main differences between these scoring systems

- page 10, fourth paragraph: in several studies and guidelines, US is considered an imaging technique with limited sentitivity in the detection of liver steatosis, since it does not reliably detect steatosis when <20-25%. The ability of US to detect liver steatosis when >10% is deducted from the results of a recent study by Ballestri et al, but these results should be confirmed. Please comment

We thank rev 2 once again for his/her comment. We have addressed this topic in the manuscript by adding the following sentences: "Traditionally, ultrasonography has been considered a technique with a low sensitivity in identifying fatty liver infiltration when less than 30% of hepatocytes are steatotic.Of note, a recent studyhas demonstrated that ultrasonography, especially when implemented with standardized measurements and semi-quantitative scores, is sensitive for an amount of steatosis as low as 10% [106] and may predict metabolic derangements and liver histology changes [106-108]. Further studies are necessary to confirm these novel findings."

- after the diagnostic section, I suggest to rearrange the layout as follow: 1. NAFLD: clinico-laboratory features; 2. NAFLD as multisystem disease; 3. NAFLD and T2D; 4. NAFLD: a large spectrum of clinical associations; 5. principles of treatment

We agree on the proposed rearrangement of the layout of the manuscript. Therefore, we have reworked the sections as suggested.

- page 14, third paragraph: the topic on NOSA is not strictly linked to IR and hyperuricemia. If the authors prefer, they should address this issue in another paragraph. Furthermore, as laboratory features, at least liver function tests, lipid profile, iron parameters should be evaluated

We thank rev 2 for his/her comment. The topic on NOSA has been moved to another paragraph entitled "Liver tests, parameters of iron metabolism and non-organ specific autoantibodies" in which we also evaluated the role of liver tests and ferritin levels. We also evaluated lipid profile as suggested by adding this topic in a new paragraph entitled "Insulin Resistance, Hyperuricemia and Atherogenic Dyslipidemia".

We have added the following sentences to the manuscript:

"NAFLD is typically characterized byatherogenic dyslipidemia featuring larger and triglyceride over-enriched circulating very-low-density lipoproteins (VLDLs), small dense low-density lipoproteins (LDLs) and low and dysfunctional high-density lipoproteins (HDLs). Of note, when NAFLD progresses to severe fibrosis and cirrhosis, dyslipidemia will apparently improve, probably owing to failing hepatic synthetic capacity [144, 156-159].

Liver tests, parameters of iron metabolism and non-organ specific autoantibodies

Liver tests are typically normal or mildly elevated in NAFLD. Gamma-glutamyltransferase (GGT) may be slightly elevated and is increasingly recognized as a marker of metabolic disturbances and cardiovascular risk [160]. Aminotransferases do not identify progressive disease, given that patients with normal liver enzymes are spared neither from NASH nor significant fibrosis [161].

The diagnostic process followed to investigate a suspect liver disease may include the determination of parameters of iron metabolism and non-organ specific autoantibodies (NOSA). Elevated serum ferritin, closely associated with hepatic iron deposition, is common in NAFLD and is strongly correlated with IR, more advanced disease and increased mortality [162-164]. Whether therapeutic strategies aimed at correcting iron metabolism may be beneficial in NAFLD remains controversial. "

- page 18, second paragraph: please specify that it's a phase 2 randomized study

We have added the phase and the design of the study as suggested.

- table 1 should be removed

We have deleted this table as requested. In its place we have now added a novel table 1.

- the manuscript should be revised for typo

We have carefully revised all the manuscript for typos.

Reviewer #3

Please adjust the grammatical and spelling errors pointed out in the manuscript. Also, define terminologies as they are encountered for the first time, hence the appropriate usage of their acronyms can follow. For example, the same way you correctly defined and abbreviated NAFLD in the abstract, you should also do the same for MetS the first time it is mentioned in the abstract. Furthermore, the first-time IR was mentioned in its acronym form on page 3, it was not spelled out; but it was later spelled out on page 5. It should be the other way around.

We have carefully revised all the manuscript for typos, grammatical and spelling errors. Moreover, we have carefully revised the acronyms throughout the manuscript.

It is not clear when you cite epidemiological figures whether you are referring to US or world populations. For example, you stated: "In the general population, the prevalence of NAFLD has been reported to widely range from 6.3% to 51% related to the different population/ethnicity evaluated as well as to the diagnostic methods utilized to assess the deposition of liver fat content [43]". Please be specific!

We thank rev 3 for his/her comment. For sake of clarity, we have reworked the sentence as follows "Epidemiological studies from around the world reported that the prevalence of NAFLD widely

ranges from 6.3% to 51% related to the different population/ethnicity evaluated as well as to the diagnostic methods utilized to assess the amount of intrahepatic fat content"

Also, please revise the statement "At variance with what was found in the general population, women with T2D are exposed to the same risk of NAFLD as men, indicating that T2D abrogates that protection from developing NAFLD which is usually provided to ladies by their hormonal profile and/or set of chromosomes [7, 14].? Please revise this phrase." I have modified this sentence to state what I think you meant. if not, please revise as it does not convey any meaningful message as it currently stands.

We thank rev 3 for raising this point. For sake of clarity, we have reworked the sentence as follows: "In the general population, women are protected from developing NAFLD owing to their hormonal profile and/or set of chromosomes. Conversely, women with T2D are exposed to the same risk of NAFLD as men, indicating that T2D abrogates such a gender-related protection[7, 14]."

On page 9, the assertion that "Steatosis, namely a minimal threshold of 5% hepatocytes containing fat droplets." – is the 5% by weight of the liver? There also seems to be a discrepancy between the above 5% hepatocytes mentioned in the manuscript and the sentence on page 10 that states "The main advantages of ultrasonography are its safety, low cost, wide availability, the overall scanning of abdominal organs and the accuracy for the diagnosis of steatosis affecting >10% of hepatocytes". This statement implies that ultrasound cannot be used for determining steatosis in less than 10% of the affected hepatocytes. Again, there is a discrepancy in relating the word "accuracy" between the statements "The main advantages of ultrasonography are its safety, low cost, wide availability, the overall scanning of abdominal organs and the accuracy for the diagnosis of steatosis affecting >10% of hepatocytes" and "The main limitations of ultrasonography are its inaccuracy in differentiating steatosis from fibrosis, the issues with morbid obese individuals, and its operator and machinery dependency".

We thank rev 3 once again for this point. We have reworked the sentences as follows:

"Steatosis, namely a minimal threshold of 5% of hepatocytes containing fat droplets in biopsy specimen, is a prerequisite for the diagnosis of NAFLD."

"The main advantages of ultrasonography are its safety, low cost, wide availability and the overall scanning of abdominal organs. Traditionally, ultrasonography has been considered a technique with a low sensitivity in identifying fatty liver infiltration when less than 30% of hepatocytes are steatotic. Of note, a recent study has demonstrated that ultrasonography, especially when implemented with standardized measurements and semi-quantitative scores, is sensitive for an amount of steatosis as low as 10% [106] and may predict metabolic derangements and liver histology changes [106-108]. Further studies are necessary to confirm these novel findings.The main limitations of ultrasonography are its inability in differentiating steatosis from fibrosis, the issues with morbid obese individuals, and its operator and machinery dependency."

The standard abbreviation or acronym for colorectal cancer is "CRC" and not "CCR".

The acronym has been corrected.

Reviewer #4: Dear Associate Editor, Thank you for sending this review. This review focus on the updates on epidemiology, pathogenesis, diagnosis and treatment of NAFLD. The manuscript is well written.

We wish to thank Rev. 4 for his/her kind comments.

Reviewer #5: The manuscript is good and suitable for publication

We wish to thank Rev. 5 for his/her kind comments.