January 29, 2014

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 6831-edited.doc).

Title: Clinical differences between alcoholic liver disease and nonalcoholic fatty liver disease

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Name of Journal: *World Journal of Gastroenterology*

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

1 Format has been updated.

→ According to the writing requirements, we reduced the number of words in the title.

Original title: Comparison of clinical features and outcomes in patients with alcoholic liver disease and patients with nonalcoholic fatty liver disease

New title: Clinical differences between alcoholic liver disease and nonalcoholic fatty liver disease

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

(1) Comments made by Reviewer 02444760: Major 1.Running title of the review, being 'Hepatitis C-related liver cirrhosis', may be unrelated to the text. Is there any mistake?

→ Thank you very much for pointing out our mistake.

Correction : Clinical differences between ALD and NAFLD

(2) Comments made by Reviewer 02444760: Major 2.According to the review, non-invasive methods, such as AST/platelet ratio index (APRI), FIB-4 index, FibroScan, and FibroTest, have been employed to assess the fibrosis stage of ALD and NAFLD. However, non-invasive method related to the hepatic steatosis of ALD and NAFLD, both of which are characterized by hepatocyte steatosis, is ignored. To the knowledge of reviewer, controlled attenuation parameter (CAP) is recently developed to measure the hepatic steatosis using a process based on transient elastography. Then the authors are encouraged to evaluate the progression in non-invasive staging of hepatic steatosis.

→ Thank you very much for your suggestion. Following your suggestion, we added the following paragraph.

Page 9, line 17 - 21 and page 10, line 1 - 5

Recently, a novel measurement method for assessing the hepatic steatosis grade was developed, which has been designated as the controlled attenuation parameter (CAP)[39]. This method is based on transient elastography and permits a simultaneous evaluation of the fibrosis stage and the steatosis grade. Studies have demonstrated the usefulness of CAP in quantitatively assessing the steatosis grade with a high accuracy[40-42]. A large cohort study found that the AUROCs of the CAP for the diagnosis of steatosis > 10%, steatosis > 33%, and steatosis > 66% were 0.79, 0.84, and 0.84, respectively, in 440 patients who underwent a liver biopsy[42]. This study also found that elevated CAP values were significantly associated with excessive alcohol intake, indicating a possible clinical application of CAP in the diagnosis and management of ALD.

Accordingly, the position of the sentence "Further validation studies are required to establish the usefulness of these non-invasive methods in patients with ALD and NAFLD." was changed from page 8, line 20 - 21 to page 10, line 6 - 7.

(3) Comments made by Reviewer 02444760: Major 3.Except for these non-invasive measurements, scoring the features of ALD and NAFLD reflects another effective method in the assessment of disease stage. For example, NAFLD Activity Score (NAS) is used to separate NAFLD from nonalcoholic steatotic hepatitis (NASH). Maddrey Discriminant Function (MDF) or Model for End-Stage Liver Disease (MELD) scoring system uncovers the risk of poor outcome in patients with alcoholic hepatitis (AH). Scoring of ALD and NAFLD, therefore, is suggested to be discussed.

→ We appreciate your helpful suggestion. Following your suggestion, we added a new Chapter (2.3.) for describing scoring systems for ALD and NAFLD.

Page 10, line 8 - 19

2.3. Scoring systems for ALD and NAFLD

Scoring systems specific for ALD or NAFLD have been developed to assess disease severity and stage and/or to predict patient outcomes. Scoring systems for ALD[43, 44] include Maddrey's discriminant function (mDF), the Glasgow Alcoholic Hepatitis Score (GAHS), the Age-Bilirubin-International Normalized Ratio (INR)-Creatinine (ABIC) score, and the Lille score. Studies have demonstrated that these systems are useful in predicting the short-term survival of patients with alcoholic hepatitis[44, 45]. The Brunt score[46] and the NAFLD Activity Score (NAS)[47] are histological scoring systems for NAFLD. Studies have suggested that the NAS has an excellent ability to differentiate simple hepatic steatosis from nonalcoholic steatohepatitis (NASH)[48]. Other scoring systems for the assessment of NAFLD stage, such as the NAFLD fibrosis score and the BARD score, have shown promising results[49].

(4) Comments made by Reviewer 02444760: Minor 1. Patatin-like phospholipase domain-containing 3 (PNPLA3), especially I148M (rs738409 C/G), has bee proved to play an important role in both ALD and NAFLD. But accumulating proofs confirm that different SNPs of PNPLA3, and various genes other than PNPLA3, implicate in ALD and/or NAFLD. As a result, more information is needed to shed light on the genetic variance of ALD and NAFLD.

→ Thank you very much for your helpful suggestion. Following your suggestion, we changed the title of Chapter 3.2.5. and added the following paragraph for providing information on other genetic variances.

Original title (page 12, line 4): 3.2.5. ***Patatin-like phospholipase domain-containing 3 gene variant***

New title (page 14, line 12): 3.2.5. ***Genetic factors***

Page 15, line 12 – 24 and page 16, line 1 – 2

Recent studies have found other *PNPLA3* SNPs associated with NAFLD[85, 86]. A recent GWAS of Japanese NAFLD patients identified rs2896019 and rs381062, SNPs that the investigators suggested are associated with the NAFLD grade and/or stage[86]. Additionally, this GWAS also found *SAMM50* and *PARVB* SNPs, both of which were considered to be involved in the second hit of NAFLD, leading to a shift from simple steatosis to NASH. In contrast, a GWAS of Caucasian NAFLD patients identified *FDFT1* and *COL13A1* SNPs[87]. Another GWAS of Caucasian NAFLD patients identified *NCAN* and *PPP1R3B* SNPs, as well as a *PNPLA3* SNP (rs738409), and demonstrated that these SNPs correlate with hepatic steatosis. Furthermore, it was found that SNPs in the *NCAN*, *GCKR*, *LYPLAL1*, and *PNPLA3* genes correlated with histological lobular inflammation/fibrosis[88]. In addition, accumulated data have suggested that some SNPs, other than the *PNPLA3* SNP, may be associated with the pathogenesis of ALD. A meta-analysis revealed a close relationship between a *TNFA* SNP (rs361525) and ALD[89]. Moreover, several studies have found a higher frequency of the *CD14* -159 C/T SNP in patients with alcoholic cirrhosis than in those without this disease[76].

(5) Comments made by Reviewer 02444760: Minor 2. There seems to be ambiguous in the English expressions of text, such as 'non-invasive methods cannot differentiate simple hepatic steatosis from hepatic fibrosis' (P. 8). Plain and precise expression will be appreciated.

→ We appreciate this helpful advice. According to your advice, our manuscript was proofread by an English language editing service, American Journal Experts and it has been improved. The sentence (page 8) has been changed as follows:

Original sentence (page 8, line 18 – 20): Generally, non-invasive methods cannot differentiate simple hepatic steatosis from hepatic fibrosis, but can assess the degree of hepatic fibrosis.

Revised sentence (page 9, line 14 – 16): Generally, transient elastography cannot differentiate simple hepatic steatosis from mild hepatic fibrosis but can assess the degree of hepatic fibrosis.

(6) Comments made by Reviewer 00053689: 1. In general, this review manuscript is significant and comprehensive. However, the content could be condensed to make it more concise. or instance, there are some redundant outlines between sessions of [Epidemiology] and [Factors associated with disease susceptibility and progression] and also between those of [Predictors of outcomes] and [Characteristics of outcomes], which are suggested to be merged.

→ Thank you very much for the comments. However, we believe that this text structure will be accessible to readers.

(7) Comments made by Reviewer 00053689: 2. Some grammar errors in the text are needed to be cautiously corrected. For instance, the first sentence in the session of 4.3 Mortality.

→ Thank you very much for your suggestion. Following your suggestion, our manuscript has been improved by the use of an English language editing service, American Journal Experts.

(8) Comments made by Reviewer 02444976: I think that a comment on the pathological features of the two entities is warranted since the authors do mention how histology is related to outcome and also non-invasive markers of fibrosis.

→ We appreciate this thoughtful advice. According to your advice, we described the pathological features of the two entities in the Chapter (*2.1.*).

Page 7, line 5 - 11

As mentioned above, ALD and NAFLD have similar pathological spectra, from simple steatosis to liver cirrhosis, which makes confident differentiation of the two diseases difficult. However, the following histological findings are helpful in the differential diagnosis[7]. The fatty degeneration of liver cells occurs to a greater degree in NAFLD than in ALD. In contrast, inflammatory cell infiltration is more pronounced in ALD than in NAFLD. Furthermore, venous or perivenular fibrosis, phlebosclerosis, and (less commonly) lymphocytic phlebitis are more common in ALD than in NAFLD.

Accordingly, we rewrote the following sentences.

Original sentences (page 7, line 5 - 8): Differentiation between ALD and NAFLD is occasionally difficult, since both diseases have several similar clinical features, such as hepatic steatosis. The diseases can usually be differentiated by taking a history of a patient's alcohol intake, combined with laboratory and imaging examinations;

Revised sentences (page 7, line 12 - 14): Clinically, the differentiation between ALD and NAFLD is usually performed by taking a history of a patient's alcohol intake combined with laboratory and imaging examinations;

(9) Comments made by Reviewer 02444976: The authors state in the conclusion that NAFLD is a disease caused by an addiction to food. I think this is oversimplifying the issue and suggests that the patients with NAFLD are guilty for developing the disease.

→ Thank you very much for the great advice. Following your advice, we deleted the term "addiction". Alternatively, we use the words "unhealthy lifestyle habits".

Original sentence (page 21, line 5 - 7): Both liver diseases are based on "addictions", one to alcohol and the other to food, with both likely to be serious health problems in the future.

Revised sentence (page 24, line 12 - 14): Both liver diseases are generally related to unhealthy lifestyle habits, including excessive alcohol and food intake, and both are likely to be serious health problems in the future.

(10) Comments made by Reviewer 00742517: 1.The title is: Comparison of clinical features and outcomes in patients with alcoholic liver disease and patients with nonalcoholic fatty liver disease. But the running title is : Hepatitis C-related liver cirrhosis. Please keep in consistent.

→ Thank you very much for pointing out our mistake. The running title was incorrect.

Correction : Clinical differences between ALD and NAFLD

(11) Comments made by Reviewer 00742517: 2.Please revise the format of the full text. For example:keep text-align :justify.

→ Thank you very much for your advice. Format has been updated.

(12) Comments made by Reviewer 00742517: 3.Please ask someone familiar with English language to help you to rewrite the paper. Page2 Abstract:Alcoholic liver disease (ALD) and nonalcoholic fatty liver disease (NAFLD) are worldwide, serious health problems. Revised to "Alcoholic liver disease (ALD) and nonalcoholic fatty liver disease (NAFLD) are serious health problems worldwide" would be better. Page4: Reflecting modern overnutrition, 1.46 billion adults worldwide were estimated to have a body mass index (BMI) of 25 kg/m2 or higher in 2008[4] Page4: ALD and NAFLD differ from each other in many characteristics, ranging from differences in molecular biology to clinical aspects. Revised to "ranging from differences in clinical features to outcomes" would be better. Page 5:We here therefore comprehensively characterize ALD by comparing its clinical features and outcomes with those of NAFLD. Page 5: A study from the USA of patients hospitalized for alcohol-related conditions found that the peak prevalence was observed at ages 45–69 years[15].

→ We appreciate your kind advice. According to your advice, the sentences have been improved. Furthermore, our manuscript was proofread by an English language editing service, American Journal Experts and it has been improved.

1. Original sentence (Abstract, line 1 - 2): Alcoholic liver disease (ALD) and nonalcoholic fatty liver disease (NAFLD) are worldwide, serious health problems.

Revised sentence (Abstract, line 1 - 2): Alcoholic liver disease (ALD) and nonalcoholic fatty liver disease (NAFLD) are serious health problems worldwide.

2. Original sentence (page 4, line 10 - 12): Reflecting modern overnutrition, 1.46 billion adults worldwide were estimated to have a body mass index (BMI) of 25 kg/m2 or higher in 2008[4].

Revised sentence (page 4, line 10 - 12): . An estimated 1.46 billion adults worldwide had a body mass index (BMI) of 25 kg/m^2 or higher in 2008, which reflects modern overnutrition[4].

3. Original sentence (page 4, line 19 - 20): ALD and NAFLD differ from each other in many characteristics, ranging from differences in molecular biology to clinical aspects.

Revised sentence (page 4, line 19 - 20): ALD and NAFLD differ from each other in many characteristics, ranging from differences in clinical features to patient outcomes.

4. Original sentence (page 5, line 1 - 3):We here therefore comprehensively characterize ALD by comparing its clinical features and outcomes with those of NAFLD.

Revised sentence (page 5, line 2 - 3): Therefore, in this review, we comprehensively characterized ALD by comparing its clinical features and outcomes with those of NAFLD.

5. Original sentence (page 5, line 18 - 19):A study from the USA of patients hospitalized for alcohol-related conditions found that the peak prevalence was observed at ages 45–69 years[15].

Revised sentence (page 5, line 18 - 19): A study from the USA revealed that the peak prevalence of hospitalization for alcohol-related conditions was between 45 and 69 years of age[15].

(13) Comments made by Reviewer 00742517: 4. Page 7: About the ALD/NAFLD index (ANI):The author should point the limitation. it is not suitable for end-stage liver disease and cirrhosis as patients in this stage all have elevated MCV and AST/ALT.

→ Thank you very much for your suggestion. According to your suggestion, we described the limitation of the ANI.

Page 7, line 22 and page 8, 1 - 2
However, the ANI may be less reliable in patients with end-stage liver disease because these patients frequently have an elevated MCV and an increased AST/ALT ratio[25].

(14) Comments made by Reviewer 00742517: 5. Page 8: The author just show the accuracy of many fibrosis indices using AUROCs. But the author also should show the sensitivity and specificity of fibrosis indices such as Fibrotest.

→ We appreciate the great advice. According to your advice, we added the sensivity and specificity for each index, if described in the cited references, in Chapters *2.1. Differentiation* and *2.2. Assessment of disease stage*. Furthermore, we corrected some errors in AUROCs.

Original sentence (page 7, line 12 - 14): This index showed high diagnostic accuracy, with the area under the receiver operating characteristic curve (AUROC) being 0.98 in the derivation set; and 0.974, 0.989, and 0.767 in the three validation sets.

Resived sentence (page 7, line 18 - 21): This index has exhibited a high diagnostic accuracy, with an area under the receiver operating characteristic curve (AUROC) of 0.983 (cut-off value, 0; sensitivity, 93.5%; specificity, 92.0%) in the derivation set and AUROCs of 0.974, 0.989, and 0.767 in the three validation sets.

Original sentences (page 8, line 6 - 18): The AUROC of FibroTest for diagnosis of liver cirrhosis in patients with ALD was found to be 0.95[32], while a more recent study found that the AUROCs of APRI, FIB-4 index, and FibroTest for liver cirrhosis in patients with ALD were 0.59, 0.70, and 0.83, respectively[33]. In patients with NAFLD, the AUROCs of APRI and FIB-4 index for the diagnosis of advanced fibrosis were 0.73 and 0.82, respectively[34], and 0.82 and 0.87, respectively[35]. Another study in patients with NAFLD showed that the AUROCs of FibroTest for diagnosis of advanced fibrosis in two independent cohorts were 0.92 and 0.81[36].

Transient elastography has also been shown useful in assessing disease stage. For example, its accuracy in assessing of fibrosis stage was validated for ALD, with AUROCs of 0.94 for extensive fibrosis and 0.87 for liver cirrhosis[37]. The AUROCs of transient elastography, APRI, and FIB-4 index for diagnosis of liver cirrhosis in patients with NAFLD were 0.95, 9.75, and 0.81, respectively[38]

Revised sensentences (page 8, line 14 - 21 and page 9, line 1 - 14): The AUROC of the FibroTest for the diagnosis of liver cirrhosis in patients with ALD was 0.95 (cut-off value, 0.7; sensitivity, 91%; specificity, 87%)[32], while a more recent study found that the AUROCs of the APRI, the FIB-4 index, and the FibroTest for liver cirrhosis in patients with ALD were 0.67, 0.80, and 0.94 (cut-off value, 0.7; sensitivity, 86.6%; specificity, 86.0%), respectively[33]. In patients with NAFLD, the AUROCs of the APRI and the FIB-4 index for the diagnosis of advanced fibrosis were 0.73 and 0.80 (cut-off value, 2.67; sensitivity, 33%; specificity, 98%), respectively[34], and in another study, the AUROCs for the APRI and the FIB-4 index were 0.82 (cut-off value, 1; sensitivity, 67%; specificity, 81%) and 0.87 (cut-off value, 3.25; sensitivity, 48%; specificity, 95%), respectively[35]. Another study in patients with NAFLD indicated that the AUROCs of the FibroTest for the diagnosis of advanced fibrosis in two independent cohorts were 0.92 (cut-off value, 0.7; sensitivity, 25%; specificity, 97%) and 0.81(cut-off value, 0.7; sensitivity, 25%; specificity, 99%), respectively[36].

Transient elastography has also been shown to be useful in assessing the disease stage. For example, the accuracy of this method in assessing the fibrosis stage was validated for ALD, with AUROCs of 0.94 (cut-off value, 11.60 kPa; sensitivity, 87%; specificity, 89%) for advanced fibrosis and 0.87 (cut-off value, 22.70 kPa; sensitivity, 84%; specificity, 83%) for liver cirrhosis[37]. The AUROCs of transient elastography, the APRI, and the FIB-4 index for the diagnosis of advanced fibrosis in patients with NAFLD were 0.93 (cut-off value, 9.6 kPa; sensitivity, 63.6%; specificity, 83.7%), 0.74 (cut-off value, 0.5; sensitivity, 65.1%; specificity, 72.3%), and 0.80 (cut-off value, 2.67; sensitivity, 20.6%; specificity, 95.5%), respectively[38].

(15) Comments made by Reviewer 00742517: 6.Page 10 Paragraph 1:Risks for the ALD are associated with alcohol type, quantity, drinking patterns and so on.The author should detail these factors.

→ Thank you very much for your helpful comments. Following your suggestion, we added the following sentences.

Page 12, line 3 - 7

Drinking patterns are also factors associated with ALD. Studies have demonstrated that daily or near-daily heavy drinking, not episodic or binge drinking, is closely associated with ALD development[18, 60]. Moreover, it was demonstrated that alcohol intake outside of mealtimes and the intake of multiple, different beverages increase the risk of developing ALD[18].

Accordingly, we revised Table 2 (Environmental factors associated with disease susceptibility).

(16) Comments made by Reviewer 00742517: 7.Page 10 section :Host factors. This section should include factors such as insulin resistance and metabolic syndrome.

→ We appreciate your great advice. Following your advice, we changed the title of Chapter 3.2.3.and revised the following sentence for describing insulin resistance and metabolic syndrome.

Original title (page 11, line 7): 3.2.3. ***Obesity and type 2 diabetes***

New title (page 13, line 11): 3.2.3. ***Obesity, metabolic syndrome, and type 2 diabetes***

Original sentence (page 11, line 10 - 13): Little is known about the relationship between type 2 diabetes and ALD development and progression, whereas type 2 diabetes was found highly associated with NAFLD development[69] but not NAFLD progression[68].

Revised sentence (page 13, line 15 - 21): Insulin resistance is a key factor in the development of metabolic syndrome[69] and is largely responsible for the development of type 2 diabetes. Recent studies have demonstrated the close relationship between ALD and insulin resistance[70] and have suggested that metabolic syndrome and type 2 diabetes are associated with the development of ALD[71]. In contrast, insulin resistance or metabolic syndrome and type 2 diabetes were found to be highly associated with NAFLD development[22, 71, 72] but not NAFLD progression[68].

Accordingly, we revised Table 2 (metabolic syndrome and type 2 diabetes).

(17) Comments made by Reviewer 00742517: 8.Page 19 Section 6.3. Mortality and causes of death survival parameters were similar in patients with decompensated alcoholic and hepatitis C-related cirrhosis. What is the survival parameters? 5-year survival rates or others?

→ Thank you very much for the comments. We revised the sentence to be more precise.

Original sentence (page 19, line 9 - 11): In an analysis, survival parameters were similar in patients with decompensated alcoholic and hepatitis C-related cirrhosis, indicating that the cause of liver disease did not affect survival [71].

Revised sentence (page 22, line 15 - 17): In a study of the survival of patients with decompensated alcoholic and hepatitis C-related cirrhosis, a multivariate analysis revealed that the cause of liver disease did not affect survival[91].

3 References and typesetting were corrected.

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

Sincerely yours,

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