Format for ANSWERING REVIEWERS

August 23, 2014

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 11731-edited 2 (9).doc).

Title: Characteristics of hepatocellular carcinoma in cirrhotic and non-cirrhotic non-alcoholic fatty liver disease

Author: Christopher Leung, Sern Wei Yeoh, Desmond Patrick, Shara Ket, Kaye Marion, Paul Gow, Peter W Angus

Name of Journal: *World Journal of Gastroenterology*

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Thank-you for reviewing our paper. We feel that after the changes suggested by the reviewers that it is now a stronger, more robust manuscript that would be of broad interest to the readership of the World Journal of Gastroenterology.

The manuscript has been improved according to the suggestions of the reviewers:
(1) The appropriate "Column" of this manuscript has been listed as "Original article"

(2) Reviewer code 00000456 and 02444760 have asked for more exclusion criteria re: other causes of cirrhosis which we have added, as well as formal definitions for alcohol consumption.

Thank-you for the comment. We agree that more specific exclusion criteria should be detailed and have included this on page 7, paragraph 2 with the sentence: "cleared or chronic hepatitis B (defined as having detectable hepatitis B core antibody, with or without positive surface antigen), chronic hepatitis C, Wilson's disease, haemochromatosis, autoimmune hepatitis, alpha1-antitrypsin deficiency, cystic fibrosis, primary biliary cirrhosis, primary sclerosing cholangitis and other chronic biliary tract diseases and other hepatic malignancies had been excluded by relevant blood tests and/or liver histology. Patients were excluded if they had an alcohol intake of over 140g weekly for men and 70g weekly for women."

(3) Reviewer code 00000456 has asked us to comment on the use of BMI rather than waist circumference as a measure of NAFLD risk.

Thank-you for the suggestion. Waist circumference is indeed an important measure of NAFLD risk. Unfortunately waist circumference was not readily available in our retrospective data. We have included this statement on page 8, paragraph 1: "Obesity was measured via body mass index (BMI) instead of waist circumference which was not recorded in a majority of patients."

(4) Reviewer code 00000456 has asked us to reference both Asia-Pacific NAFLD guidelines.

Thank-you for this comment given the importance of local guidelines. We have done this and added

the sentence to page 7, paragraph 2: "Patients were included if they had characteristic radiological or histological features of NAFLD as recommended in Asia-Pacific guidelines[23]"

(5) Reviewer code 00000456 has asked us to comment on recent data in regard to HCC development via adenomas.

Thank-you for this suggestion. We have now made a comment as suggested, on page 14, paragraph 1, as follows: "Moreover, there is no way to establish a clear causal link between steatosis and carcinogesis in all these patients. Some may have been patients who developed sporadic HCC in whom the presence of hepatic steatosis may have been coincidental. Others may have had HCC derived from a pre-existing adenoma, since there is increasing literature postulating that the metabolic syndrome may drive malignant transformation of adenomas[44]."

(5) Reviewer code 00000456 has asked for references to other international (i.e. Italian guidelines).

Thank-you for the suggestion. We have now done this and cited the summary by Nascimbeni et al in reference 24. See page 7, paragraph 2, where we have added: "These diagnostic criteria broadly concur with those in International (Chinese, Italian, European and American) guidelines as summarised by Nascimbeni et al [24]"

(6) Reviewer code 00000456 has asked us to trim the discussion about alpha-fetoprotein.

Thank-you and we have now trimmed the discussion regarding this. Please see page 15, paragraph 1, which is now substantially shorter.

(7) Reviewer code 00000456 has asked us if any patients were of Asian race and therefore need altered BMI criteria for overweight and obesity.

Thank-you for this important comment given the different BMI limits for the Asian population. In fact, we did not have any Asian patients in this cohort and have added a sentence to this effect. Please see page 8, paragraph 1, where we have added: "No patients were of Asian background (in whom altered BMI cut-offs for overweight or obesity would have otherwise applied)"

(8) Reviewer code 00000456 has asked us to comment on the proportion of cirrhotic patients diagnosed by routine screening alone rather than symptoms compared with non-cirrhotic patients.

Thank-you for this question. In fact, a larger proportion of non-cirrhotics compared with cirrhotics presented with symptoms or had HCC found incidentally when a scan was performed for other reasons. This likely, as the reviewer has rightly stated, contributed to the more advanced disease seen in non-cirrhotic patients. We have addressed this on page 10, paragraph 3, with the sentences: "Twenty seven (60%) cirrhotic patients were diagnosed while asymptomatic by scheduled screening, with 9 (20%) diagnosed due to symptoms of hepatic decompensation and 10 (20%) diagnosed incidentally when being imaged for other reasons. Four (50%) non-cirrhotic patients were diagnosed due to symptoms and in 4 it was found incidentally."

(9) Reviewer code 00000456 has asked us to stratify non-cirrhotics into two groups: those with or without non-alcoholic steatohepatitis (NASH), to answer "is simple steatosis (as opposed to non-cirrhotic NASH) a risk factor for HCC development in the present series?"

Thank-you for this important question. Though there were only 8 non-cirrhotics with HCC in our study, none of them had simple steatosis. So all had a mild degree of histological inflammation. We have now added a line in the manuscript detailing this. See page 9, paragraph 2: "Two were

stage 0 with NAFLD grade 1; and 4 were stage 1-2 with NAFLD grade 2. As such, all non-cirrhotic patients had some degree of inflammation- there were none with just simple steatosis (grade 0) in our cohort." This would be an interesting question to explore in further research with a longitudinal prospective study comparing two cohorts with the above conditions, analyzing for the incidence of HCC over time.

(10) Reviewer code 02098400 has asked us to further explain figure legends

Thank-you for this comment. We changed the legends as follows;
Figure 1: BMI at diagnosis of HCC demonstrating a high rate of overweight/obesity.
Figure 2: Prevalence of number of risk factors for HCC– overweight or obesity, diabetes, hypertension and dyslipidaemia. Notably 34% of patients had less than 2 risk factors."

(11) Reviewer code 02098400 has asked us to define F0-F4:

Thank-you for this comment. We have replaced this terminology throughout the manuscript with stage 0 to stage 4 of fibrosis according to Brunt criteria.

(12) Reviewer code 02098400 has asked us to show a clinical comparison between NAFLD cirrhotics and cryptogenic cirrhotics to prove that the inclusion of cryptogenic cirrhotics was appropriate.

Thank-you for this suggestion. We have done so in Table 1. Also on page 9, paragraph 4, we have added: "Demographic and risk factor profiles of patients with NAFLD associated cirrhosis and cryptogenic cirrhosis were not statistically different, except for a higher prevalence of hypertension in the former cohort (Table 1)"; and on page 12, paragraph 1: "Indeed, in our cohort, patients with NAFLD cirrhosis had similar demographic and risk factor profiles to those with cryptogenic cirrhosis."

(13) Reviewer code 02444760 has asked us to make an acknowledgement of the male preponderance in the studied cohort, in addition to the small sample size, as a limitation of the study:

Thank-you for this observation. We have done this on page 15, paragraph 2, by adding "and a significant bias toward males".

(14) The editor has asked us to provide the tables "in Excel version"

Thank-you. This has been done to the tables.

(15) The editor has asked us to provide the figures as "decomposable figures" using PowerPoint.
Thank-you. This has been done and they can now be manually edited when double-clicked.

(16) The editor has asked us to add a 'core tip' beneath the abstract

Thank-you. We have done so and added this to page 4 as follows:
"Our study confirms that hepatocellular carcinoma can occur in non-cirrhotic non-alcoholic fatty liver disease, the incidence of which is rising worldwide.

Moreover, these cancers were found to be significantly larger and more likely to be beyond Milan criteria for liver transplantation than those occurring in cirrhotic patients.

Further research is needed to identify clinical risk factor profiles predisposing to cancer

development in patients with non-alcoholic fatty liver disease such that screening if implemented can be appropriately targeted."

(17) The editor has asked us to add a "Comments" section below "Acknowledgements"

Thank-you. We have done so on page 16 as follows:

"Background
The prevalence of non-alcoholic fatty liver disease (NAFLD) is rising worldwide, especially in industrialised countries such as Australia. This condition can progress to cirrhosis and the development of hepatocellular carcinoma (HCC). Furthermore, the condition of 'cryptogenic cirrhosis' is often thought to represent end stage-NAFLD.

Research frontiers
Basic science research has elucidated pro-carcinogenic mechanisms by which NAFLD could cause HCC in the absence of cirrhosis. At present, however, no guidelines recommend screening for HCC in non-cirrhotic NAFLD patients.

Innovations and breakthroughs
Of concern there have been increasing reports of HCC occurring in non-cirrhotic NAFLD internationally over the last decade. This phenomenon however has not been described yet in an Australian cohort. Diabetes and obesity have been found to be independent risk factors for HCC development in NAFLD, but further research is needed to define such risk factors in specifically non-cirrhotic NAFLD cohorts that could guide cost-effective HCC screening.

Applications
This study reaffirms that HCC can develop in non-cirrhotic NAFLD, but could not identify particular risk factor profiles differentiating such patients from cirrhotic patients who develop HCC. Non-cirrhotic patients however had larger tumours at diagnosis than cirrhotic patients. This study thus underlines the need for further research into HCC risk factors amongst non-cirrhotic NAFLD patients, such that future HCC screening guidelines may take such patient groups into consideration in a cost-effective and targeted manner."

(18) Reviewer code 00000456 has asked us to comment on the incidence of cholangiocarcinoma in NAFLD.

Thank-you for this important comment. None of our patients had cholangiocarcinoma and we make this point now on page 13, paragraph 2: "Also importantly, none of these patients with HCC had cholangiocarcinoma."

(19) English has been optimized throughout the manuscript.

(20) Reviewer code 02444760 has asked us to analyse the impact of histological inflammation on non-cirrhotic HCC pathogenesis in our cohort, using the components of the NAFLD Activity Score (NAS).

The NAS score was not formally reported on all samples, but instead, we have used the NAFLD grade, which grades inflammation proposed by Brunt et al (see reference 25- of note, the same author was one of the architects of the NAFLD activity score). Importantly, we have found no statistically significant difference in the distribution of NAFLD grade between cirrhotics and non-cirrhotics who develop HCC. Please refer to Table 2.

Thank-you for all the reviewer's comments. All suggestions have been attended to.

Thank you also once again for considering our manuscript in the *World Journal of Gastroenterology*.

Yours sincerely,

Christopher Leung
Liver Transplant Unit, Austin Hospital, 145 Studley Road, Heidelberg, Victoria, Australia, 3084
chris.leung@y7mail.com
Phone: +614 3 9496 5000
Facsimile: +614 3 9496 3487