

Reviewer #1:

1.1 As we can see from the data, the authors seem to analyze a complete data set. Are patients with missing data being excluded?

Response 1.1: We thank the reviewer for taking the time and effort to provide comments to improve the quality of our manuscript. Indeed, all patients included in this study had available histopathological and laboratory data for analysis. Inclusion and exclusion criteria have been edited for clarity and can be found in the first paragraph of the Materials and Methods on pages 6-7 of the revised manuscript.

1.2 As the authors stated, "Given the specialized setting of the National Institutes of Health, this population may have a higher prevalence of cirrhosis than the typical primary care setting", The analyzed population selected by the authors and the popularizing population of the study results are not consistent. The two groups of patients have different backgrounds, whether this will affect the study results?

Response 1.2: We appreciate the reviewer's comment. As mentioned in our study limitations (pages 12-13 of the revised manuscript), the study population is reflective of the patient population followed at the National Institutes of Health which is a referral center. However, in our study population the average Ishak fibrosis score was 2.4 (described in the first paragraph of the Results section on page 9) and our sample size is large (1,027 patients); therefore, we consider that are findings can be of potential use when applied to the general population.

1.3 The author chose pre-treatment liver biopsies as the gold standard, which seemed to eliminate the effects of drug intervention. However, the reality is that the vast majority of patients will be given antiviral drugs. Please explain the impact of antiviral drugs on the study results and the applicability of the study conclusion.

Response 1.3: We thank the reviewer for their comment. However, we intentionally selected untreated patients as some therapies used for viral hepatitis over the course of the study can result in bone marrow suppression (most notably Interferon-based therapies) which would significantly affect our results. In addition, untreated patients represent the majority of patients seen by primary care providers and therefore this analysis can provide information on when patients should be referred to specialized care for optimal management. We have also clarified in the methods section (page 7, lines 163-164 of the revised manuscript) that laboratory values were obtained prior to any treatment initiation.

1.4 The purpose of the study was to screen for cirrhosis(F4) rather than advanced liver fibrosis(F3). I suggest that the author can use advanced fibrosis(F3) as the research standard, which is more therapeutic or preventative.

Response 1.4: We thank the reviewer for this comment. We agree that advanced fibrosis is a timepoint in the natural history of liver disease which is relevant, as appropriate management at that point might prevent the development of cirrhosis. However, the intent of our study was to identify which individuals are more likely to require additional health care resources for the management of their liver disease. As described in the AASLD practice guidance for HBV (Terrault et al. Update on prevention, diagnosis, and treatment of chronic hepatitis B: AASLD 2018 hepatitis B guidance. *Hepatology*. 2018;67(4):1560-1599. doi:10.1002/hep.29800) and HCV (Ghany et al AASLD-IDSA Hepatitis C Guidance Panel. Hepatitis C Guidance 2019 Update: AASLD-IDSA Recommendations for Testing, Managing, and Treating Hepatitis C Virus Infection. *Hepatology*. 2020;71(2):686-721. doi:10.1002/hep.31060), as well as EASL and APASL guidelines for hepatocellular carcinoma screening (EASL Clinical Practice Guidelines: Management of hepatocellular carcinoma *J Hepatol*. 2018;69(1):182-236. doi:10.1016/j.jhep.2018.03.019 and Omata et al. Asia-Pacific clinical practice guidelines on the management of hepatocellular carcinoma: a 2017 update. *Hepatol Int*. 2017;11(4):317-370. doi:10.1007/s12072-017-9799-9), cirrhosis has important ramifications in terms of management when treatment and cancer screening are considered.

1.5 There are gender differences in platelet count and function. Please analyze the impact of gender differences on the results.

Response 1.5: We thank the reviewer for this suggestion. While we agree that gender specific cut-off values for ALT and AST are well recognized and used in clinical practice, this approach is not applied to all laboratory markers. Platelet counts can indeed vary based on gender, however regular standard ranges available to clinicians do not discriminate by sex. For the purpose of our study, in order to obtain a simple single marker for identifying cirrhosis, we evaluated a single cut-off value for all genders, in the same line of thought as other non-invasive markers of fibrosis.

Reviewer #2:

2.1 Although the idea of performing the study is interesting, the usage of platelet count in decision-making for the treatment and management of patients with HCV seems to be irrelevant. All treatment-naïve and treatment-experienced patients aged ≥ 3 years with chronic hepatitis C should be considered for DAA therapy

Response 2.1: We thank the reviewer for taking the time to review our manuscript and to provide comments and suggestion to improve our piece. In response to this comment, while all major societies have recommended DAAs for all patients and this has been applied with ease in the developed world, resource limited areas have had difficulty with access to treatment such as DAAs. Thus, the access to HCV DAA therapy is not universal (In Mongolia or countries on the African continent) and patients with more advanced disease need to be prioritized for treatment. Thus, the findings highlighted in this manuscript can assist primary care providers with early identification and triage for therapy. In addition, patients with cirrhosis require additional medical attention (hepatocellular carcinoma screening and potential management of portal hypertension) and also need to be identified.

2.2 The major problem concerns methodology: What were the exclusion criteria (apart from HIV infection) for the study? There could have been patients with concomitant diseases: acute (viral) infectious disease, onco-hematological diseases, which could make the results of platelet count unreliable (as the results within two months of liver biopsy were utilized for analysis).

Response 2.2: We thank the reviewer for this comment and apologize for the lack of clarity in our methods section. We have now clarified the inclusion and exclusion criteria of our study, which can be found on pages 6-7 in the revised Methods section. Briefly, blood draws were performed prior to liver biopsy and initiation of any anti-viral treatment. Patients with multiple concomitant liver diseases were excluded and chronicity (>6 months) of viral hepatitis was confirmed. In addition, patients with severe systemic disease were excluded from participation in protocols.

Reviewer #3:

3.1 This manuscript is aimed to find a very simple one-component marker to differentiate patients with chronic hepatitis B/C/D without cirrhosis from those with cirrhosis. The result is not very surprising, but for the first time, platelet count has been validated as a very useful marker that can be applied in low-cost situations. The study is well designed, the statistics is fine, the description and the interpretation is sound.

Response 3.1: We would like to thank the reviewer for taking the time and effort to review our manuscript and are appreciative of the comments.

Reviewer #4:

4.1 This simple preliminary test is useful in making clinical decisions and in identifying areas of specialized care. However, as numerous studies have shown, it lacks the necessary diagnostic specificity and sensitivity. I see no originality in the presented study.

Response 4.1: We thank the reviewer for taking time to review our manuscript and appreciate the opportunity to respond to their comments. We agree that thrombocytopenia is indeed well described in cirrhosis, and previous studies have attempted to address a clear cut-off value. This study represents one of the largest North American cohorts and, contrary to previous cohorts focusing on HCV (Cheung RC, Currie S, Shen H, et al. Can we predict the degree of fibrosis in chronic hepatitis C patients using routine blood tests in our daily practice?. *J Clin Gastroenterol*. 2008;42(7):827-834. doi:10.1097/MCG.0b013e318046ea9a and Renou C, Muller P, Jouve E, et al. Relevance of moderate isolated thrombopenia as a strong predictive marker of cirrhosis in patients with chronic hepatitis C virus. *Am J Gastroenterol*. 2001;96(5):1657-1659. doi:10.1111/j.1572-0241.2001.03830.x), is not limited to one subtype of viral hepatitis. Thus, the utility of this manuscript is that it provides aggregate results related to all of the chronic viral hepatitis infections (hepatitis B, C, and D), which provides ease of identification for clinical care providers (including primary care providers), and also individual analysis based on each chronic viral hepatitis infection. Our suggested cutoff has a specificity of over 80% in both our training and validation cohorts, and more importantly has a negative predictive value of 95%. In addition, as shown in Table 4, we present our sensitivity and specificity for each specific viral hepatitis demonstrating consistent results notwithstanding the etiology of liver disease.

4.2 In addition, it is limited by its retrospective design and inclusion of patients from a single institution.

Response 4.2: We thank the reviewer for this comment. We acknowledge that these are limitations of our study and we have described this in our discussion section (page 12-13, lines 325-330). However, we do believe that these limitations are partially offset by the fact this is one of the largest cohorts evaluating the thresholds of single laboratory markers to determine cirrhosis and it includes the largest number of HDV patients.

Reviewer #5:

5.1 Title section: The title "Platelet count as a Screening Tool for Compensated Cirrhosis in Chronic Viral Hepatitis" cannot show the novelty and advantages of the present study.

Response 5.1: We thank the reviewer for taking the time and effort to review our manuscript and to provide useful comments that have helped to improve the quality of our manuscript. We believe that our study serves as a confirmatory analysis to determine a threshold for non-invasive assessments of cirrhosis. As thrombocytopenia

is a known occurrence in cirrhosis we preferred not to imply that these findings were novel.

5.2 Abstract section: The methods section said "Youden's Index", but the results section did not give such data.

Response 5.2: We thank the reviewer for this comment and apologize for the omission. We have now clarified the abstract (page 3, line 59) and the methods section (page 8, lines 195-197) regarding our use of Youden's Index, as well as overall sensitivity, specificity, positive and negative predictive values for platelet counts. We have reported these findings in a table (Table 5) which has been added to the manuscript.

5.3 Except for the AUROC, what are the specificity and sensitivity?

Response 5.3: We thank the reviewer for their comment. We have now added information related to the sensitivity, specificity, positive and negative predictive values in the abstract (page 3, lines 66-67). In the main manuscript, this can be found on page 10, lines 238-239 and table 4.

5.4 What are "All other tested markers"? Please give the detailed markers?

Response 5.4: We apologize for the lack of clarity and appreciate the opportunity to clarify. The selection process for serological markers is detailed in the methods section on page 8 ("*Biomarker selection*" paragraph), the markers are listed on page 9, lines 220-221, in the "*Using a single laboratory marker to identify cirrhosis*" paragraph and in Table 2. The AUROCs of other serological markers are listed in Table 3.

5.5 "143 k/uL". Please take it as the universally used unit.

Response 5.5: We thank the reviewer for this comment and have made the requested changes to the manuscript and have modified the platelet count units to $\times 10^9/L$

5.6 "it performed equally well in the validation cohort (n=309)." What is the AUROC in the validation cohort?

Response 5.6: We appreciate this comment from the reviewer. The AUROC of the validation cohort has now been added to the abstract (page 3, line 70). In the main manuscript, all AUROCs from the validation cohort are described in Table 4.

5.7 Conclusions should be more specified to "liver cirrhosis".

Response 5.7: We thank the reviewer for their comment. We have reviewed the manuscript to ensure language consistency. As suggested, we have also revised the final concluding sentence (page 13, lines 345-348) which now reads: "This routine and widely available laboratory value may be useful in the identification of patients with cirrhosis from chronic viral hepatitis which has downstream consequences related to their treatment and management and should be further explored for these purposes.

5.8 Introduction section: Introduction should pay more attention to the role of PLT in evaluating the severity of liver diseases and portal hypertension complications, but not other contents.

Response 5.8: We thank the reviewer for their suggestion. We have now edited the introduction to focus on the non-invasive assessment of fibrosis, the use of platelet counts in evaluation of liver disease, the increasing importance of primary care in the management of viral hepatitis and the reasons why a simple screening tool is necessary in this context .

5.9 The words "expert consensus suggests that thrombocytopenia, with a laboratory cutoff value of $<150/\mu\text{L}$ " should be supported by some references.

Response 5.9: We thank the reviewer for this comment. We have replaced the above-mentioned statement and have inserted references highlighting the use of $150 \times 10^9/\text{L}$ as a commonly used threshold for thrombocytopenia (page 5, lines 108-110).

5.10 What is "Alas"? Language expression should be greatly improved.

Response 5.10: We have edited the manuscript with the aim to improve language expression and have replaced the word "Alas" with "Unfortunately" (page 6, line 123).

Reviewer #6:

6.1 The aim of this study was to assess whether platelets or other laboratory markers can be used as a simple method to identify the development of cirrhosis. This topic isn't new, and several studies have been published decades ago. On the other hand, the role of platelet count in the diagnosis of cirrhosis is very well known, and an answer to the aim of this study can be found in several articles that were published more than a decade ago. Some examples: - Cheung et al. in a series of 4462 patients of national (U.S.), multicenter, ethnically diverse cohort, found that a platelet count of $< 150/\text{L}$ can be used in routine clinical practice to exclude or confirm the presence of advanced

fibrosis [Cheung RC, Currie S, Shen H, et al. Can we predict the degree of fibrosis in chronic hepatitis C patients using routine blood tests in our daily practice? *J Clin Gastroenterol.* 2008 Aug;42(7):827-34] -Renou et al report that thrombopenia detected in patients with chronic hepatitis C constitutes an accurate marker of hepatic fibrosis and an excellent predictive noninvasive marker of cirrhosis in the absence of clinical, biological, endoscopic, or ultrasonographic signs of portal hypertension or hepatocellular failure [Renou C, Muller P, Jouve E, et al. Prevalence of moderate isolated thrombocytopenia as a strong predictive marker of cirrhosis inpatient with chronic hepatitis C. *Am J Gastroenterol.* 2001;96:1657-1659]. -Qamar et al. report that, in patients with compensated cirrhosis, thrombocytopenia (defined as a platelet count $<150\,000/\mu\text{L}$) is the most common peripheral blood alteration, occurring in almost 78% of patients [Qamar AA, Grace ND, Groszmann RJ, et al. Incidence, prevalence, and clinical significance of abnormal hematologic indices in compensated cirrhosis. *Clin Gastroenterol Hepatol.* 2009;7:689-695]. Therefore, this study lacks any interest.

Response 6.1: We would like to thank the reviewer taking the time to review our manuscript and for providing comments on how to further improve this piece. We agree that thrombocytopenia in cirrhosis is a well described entity and the cutoff of $150 \times 10^9/\text{L}$ has been previously studied. However, we do believe that our study can add to the current body of literature as it pertains to a larger cohort (1027 patients) than the studies cited above (490 in the Cheung et al. study, 110 in the Renou cohort and 213 in the Qamar study). In addition, most of the previous studies focus on HCV only whereas our cohort describes a sizeable number of both HBV and HDV patients.

6.2 On the other hand, in primary care settings, simple nonproprietary biomarkers of liver fibrosis are nowadays available. Among them, the APRI score (AST to Platelet Ratio Index) is a very simple one. It would have been of interest to know if platelet count alone was more accurate than the APRI score.

Response 6.2: We thank the reviewer for this comment. It is clear that the greater hepatology (and sometimes gastroenterology) community can rely on multiple non-invasive markers which are routinely used in specialized healthcare settings. However, in the primary care setting and in resource poor settings, these specialized non-invasive tests are often not utilized and a single marker can therefore be of greater use to clinicians. We believe that a single laboratory marker, when performing with accuracy, represents a much easier tool for primary care healthcare workers (including physician extenders). Related to the second portion of the reviewer's comments, when assessing our overall cohort, the confidence intervals for the AUROC for both APRI (Training cohort: 0.84 (0.80-0.88) and Validation cohort 0.82 (0.74-0.90)) and Fib4 (Training cohort 0.88 (0.85-0.91) and Validation cohort 0.86 (0.80-0.93)) overlap with the AUROC for platelet count alone (0.86 in the training cohort) for prediction of cirrhosis. Our findings have been added to the results section, lines 232-234 and 250-252 and the discussion section, lines 302-303.

6.3 Line 75: The journal has a worldwide readership that may be unaware of these specific U.S. programs. Therefore, it must be stated here that this are both U.S. programs and not worldwide programs.

Response 6.3: We greatly appreciate the reviewer's comment. We have now clarified that the programs mentioned are indeed US programs and have also expanded discussion related to other programs that can be found worldwide. These clarifications can be found on page 5, lines 98-100, in the introduction section of the manuscript and page 12, lines 310-312 of the discussion section of the manuscript.

6.4 Line 114: Again, please specify that this applies to U.S.

Response 6.4: We thank the reviewer for this comment. We have now added clarification, as specified in our previous response, that this is occurring in the US however we have also included references to other areas worldwide where primary care providers are increasingly involved in management of viral hepatitis.

6.5 Line 177: Why only the platelet count? The methods should have been set before knowing the results.

Response 6.5: We thank the reviewer for this comment and appreciate the opportunity to clarify. We have modified the language in the methods to specify that only the most significant factor in the training cohort was then tested in the validation cohort with the use of the same cut-off. This can be found on page 8, lines 197-200 of the revised manuscript.

6.6 Lines 243-244: it seems all the way round, since the PPV was 48%.

Response 6.6: We thank the reviewer for their comment. Notably, the strength of this analysis is the ability to rule out-cirrhosis (negative predictive value of 95%). Thus, we have provided clarification in the discussion section of this manuscript (page 11, lines 271-273) and in the conclusion (page 13, lines 343-345).

6.7 Lines 261-263: I do not agree with this statement. The Fib-4 score is very simple, it is not a proprietary biomarker and is easily obtained using a formula available also online. It should be explained why platelet count alone is better than the freely available biomarkers that incorporate platelet count in them. The APRI test is even more simple than Fib-4. The authors should have compared the accuracy of Fib-4 or APRI against platelet count.

Response 6.7: We acknowledge and appreciate the reviewer's opinion related to the use of FIB-4 and APRI. As described in response 6.2, although FIB-4 and APRI are commonly utilized within the toolbox of hepatologists and gastroenterologists, they are not routinely used by primary care providers (or physician extenders). Thus, the availability of a single routine lab test provides non-subspecialists with extra tools to with which to identify a cohort of patients with cirrhosis from chronic viral hepatitis who can further be referred to subspecialists for treatment and additional medical management (variceal and hepatocellular carcinoma assessments).

Additionally, as described in the second portion of the response to comment 6.2, when looking at our overall cohort, the confidence intervals for the AUROC for both APRI and Fib4 overlap with the AUROC for platelet count alone for prediction of cirrhosis. We have included a mention of this in the results section, lines 232-234 and 250-252. Thus, as described, our intent was to identify a single laboratory marker, namely platelet counts, which appear to perform as well as the fibrosis scores.

6.8 Lines 266-267: this is a limitation in the USA not in other parts of the world.

Response 6.8: We thank the reviewer for their comment. While we agree that this is not a limitation in all laboratories worldwide, we cite sources from other parts of the world besides the United States and both references by Lilford et al. (References 14 and 28) that pertain to studies from the United Kingdom (page 21, lines 296-297).

Reviewer #7:

7.1 Hepatitis D infection is possible only with co-infection with hepatitis B, so pure hepatitis D does not exist: there are hepatitis B without D and hepatitis B with D. I think the terminology should be corrected.

Response 7.1: We would like to thank the reviewer for taking the time to review our study and to provide comments to improve our manuscript. We agree with the reviewer and appreciate the opportunity to clarify this. We have made changes to our manuscript and refer to hepatitis D as HBV/HDV co-infection. This can be found on pages 7 and 10 (lines 148 and 248 respectively) of the revised manuscript.

7.2 You write "1028 subjects (HCV = 701, HBV = 240 and HDV = 86)", but $701 + 240 + 86 = 1027$: you have lost one subject.

Response 7.2: We thank the reviewer for identifying this technical error. We have now corrected the discrepancy in patient numbers.

7.3 AUROC should always be reported with a confidence interval.

Response 7.3: We thank the reviewer for this comment and have made the requested changes and included confidence intervals for AUROC. This can be found on page 9 (line 228) and page 10 (line 250) of the revised manuscript.

7.4 APRI index is used in hepatology for similar cases. It is also quite easy to calculate. I think it would be logical to compare the predictive characteristics of this index and yours to show which one is better.

Response 7.4: We appreciate this comment by the reviewer. While APRI and Fib-4 are commonly used by hepatologists in specialized settings, their use is not as widespread in the primary care setting. We aimed to determine whether a single marker can be of greater use to clinicians and become an asset for primary care healthcare workers. Furthermore, when looking at our overall cohort, the confidence intervals for the AUROC for both APRI (Training cohort: 0.84 (0.80-0.88) and Validation cohort 0.82 (0.74-0.90)) and Fib4 (Training cohort 0.88 (0.85-0.91) and Validation cohort 0.86 (0.80-0.93)) overlap with the AUROC for platelet count alone (0.86 in the training cohort) for prediction of cirrhosis. These findings have been added to the results section, lines 232-234 and 250-252 and lines 302-303 in the discussion section. We therefore feel confident in promoting the use of platelet counts for screening for cirrhosis in the primary care setting.

7.5 According to Table 4, the platelet count in the blood has a high negative predictive value and a low positive predictive value. This means that if a patient has a platelet count of more than 140, he/she is almost guaranteed to have no cirrhosis, but if it is less, then this means practically nothing. This information should be added to the Discussion and in the same place it should be described what to do with patients with platelets below 140: should they be subjected to other, more reliable methods for detecting compensated cirrhosis (Fibroscan and others) or not. This should also be specified as a limitation of your method.

Response 7.5: We thank the reviewer for their comment. We are in agreement and have made changes to the discussion and highlighted that one of the main uses of platelet count of 140 is to rule-out cirrhosis (page 11, lines 271-273 and page 13, lines 343-345). In the setting of thrombopenia, referral to a specialist is recommended before determining whether to pursue additional non-invasive methods or a liver biopsy. We did not suggest an algorithm for patients potentially incorrectly classified as this would be beyond the scope of our study.

Science editor

Scientific quality: This manuscript is a Retrospective Study, and it does not reach the publication standard of the WJG. (1) Classification: Grade B, three Grades C, two

Grades D, and Grade E; (2) Summary of the Peer-Review Report: Reviewer 03024263 pointed out that there is no originality in the presented study. In addition, it is limited by its retrospective design and inclusion of patients from a single institution; Reviewer 02663375 pointed out that This topic isn't new, and several studies have been published decades ago. On the other hand, the role of platelet count in the diagnosis of cirrhosis is very well known, and an answer to the aim of this study can be found in several articles that were published more than a decade ago. 2 Language quality: Classification: Five Grade A, Grade B, and Grade C. 3 Recommendation: Rejection.

Response: We would like to thank the reviewers for their comments and the editor for this opportunity to review our manuscript and present a revised version. While we agree that thrombocytopenia in cirrhosis is a well described finding, we present data from the largest North-American cohort which comprises patients with three types of viral hepatitis. Furthermore, we were able to evaluate multiple cut-off points for platelet counts and we present a different perspective on the widely used value of $150 \times 10^9/L$ and suggest $140 \times 10^9/L$ can be reliable used as well and potentially validated in other future cohorts. In addition we remain hopeful that our findings will enable physicians in the primary care setting to better identify which patients require referral to specialized healthcare for management of cirrhosis.