Dear Editor and Reviewers,

Thank you for your letter and for the reviewers' comments concerning our manuscript entitled "Declining diagnostic accuracy of non-invasive fibrosis tests is associated with elevated alanine aminotransferase in chronic hepatitis B". The comments were all valuable and have been very helpful for revising and improving our paper as well as for providing important guiding significance to our research. We have studied the comments carefully and have made corrections that we hope will meet with approval.

The main corrections in the manuscript and the responses to the editor's comments are as follows:

1. A short running title was provided.

2. The postcode was included.

3. Grant approval was provided. Please refer to the attached files (41009-Funding Agency Copy of Approval Documents).

4. The format of the informed consent form was provided. Please refer to the attached files (41009-Signed Informed Consent Documents).

5. The telephone number was included.

6. An Audio Core Tip was offered (Please check the attached files (41009-Audio Core Tip).

7. For Figure 1, we have provided a decomposable figure.

8. The abbreviations were defined in all figure legends.

Responses to Reviewers

To Reviewer 1(Reviewer's code: 02811953):

1. Responses to comment (The major concern is that the authors actually had investigated the relationship between the ALT and fibrosis in patients with CHB. Since they did not alter the levels of the ALT and liver functions, they could not conclude that it was the effect of ALT on fibrosis. This should be corrected. They are actually looking for a correlation, not effect).

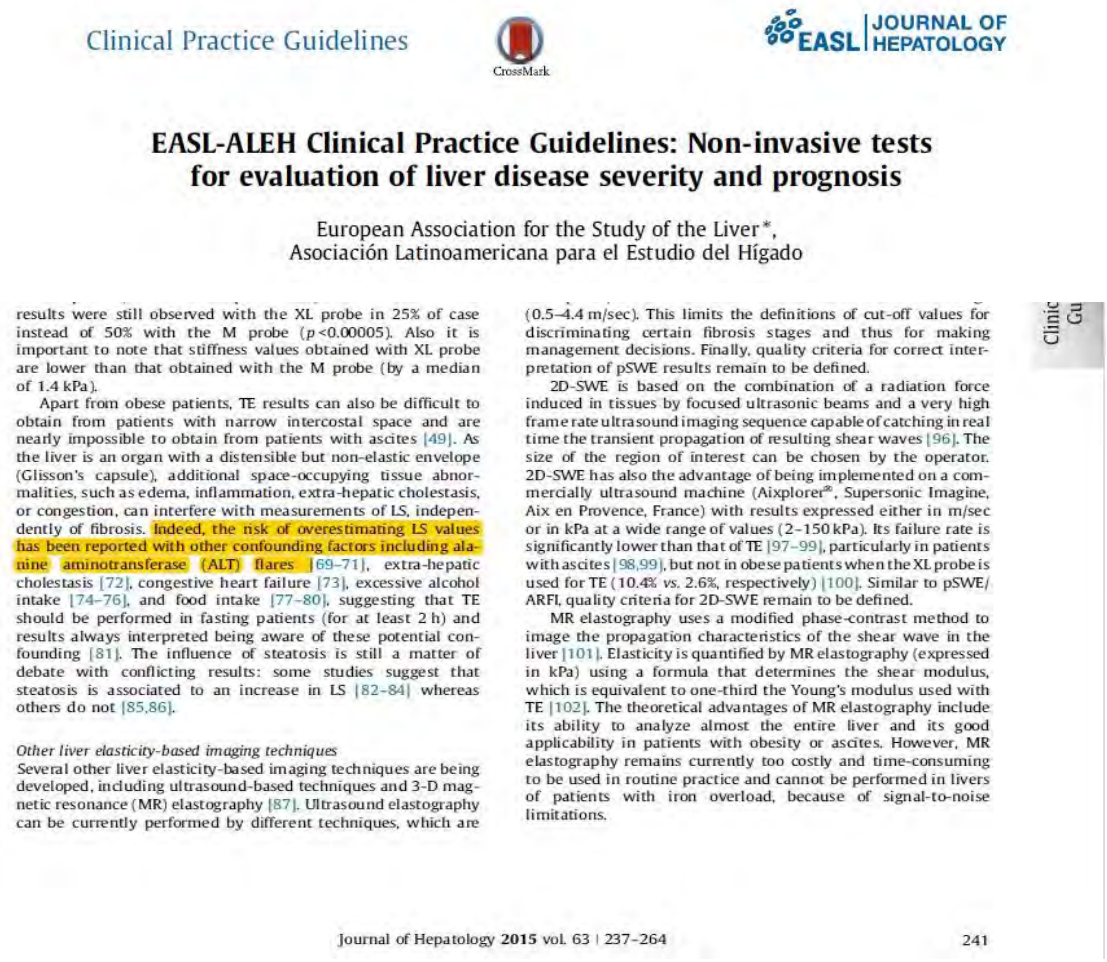
Responses:

(1) Considering the Reviewer's suggestion, first we have added the relationship between the ALT levels and liver functions in our manuscript results.

(2) Undoubtedly, a relationship exists between ALT and liver functions or fibrosis in patients with CHB. Moreover, we found that alterations to the serum ALT level could influence the diagnostic performance of non-invasive fibrosis tests, including the fibrosis test scores, AUROCs and diagnostic accuracy. We believe that a simple correlation cannot explain these results.

(3) According to the EASL-ALEH Clinical Practice Guidelines, ALT is a confounding factor for liver stiffness (LS) measurement and can influence LS (Reference 1).

Reference 1



Clinical Practice Guidelines

JOURNAL OF HEPATOLOGY

EASL-ALEH Clinical Practice Guidelines: Non-invasive tests for evaluation of liver disease severity and prognosis

European Association for the Study of the Liver*,
Asociación Latinoamericana para el Estudio del Hígado

results were still observed with the XL probe in 25% of case instead of 50% with the M probe ($p<0.00005$). Also it is important to note that stiffness values obtained with XL probe are lower than that obtained with the M probe (by a median of 1.4 kPa).

Apart from obese patients, TE results can also be difficult to obtain from patients with narrow intercostal space and are nearly impossible to obtain from patients with ascites [49]. As the liver is an organ with a distensible but non-elastic envelope (Glisson's capsule), additional space-occupying tissue abnormalities, such as edema, inflammation, extra-hepatic cholestasis, or congestion, can interfere with measurements of LS, independently of fibrosis. Indeed, the risk of overestimating LS values has been reported with other confounding factors including alanine aminotransferase (ALT) flares [69–71], extra-hepatic cholestasis [72], congestive heart failure [73], excessive alcohol intake [74–76], and food intake [77–80], suggesting that TE should be performed in fasting patients (for at least 2 h) and results always interpreted being aware of these potential confounding [81]. The influence of steatosis is still a matter of debate with conflicting results: some studies suggest that steatosis is associated to an increase in LS [82–84] whereas others do not [85,86].

Other liver elasticity-based imaging techniques

Several other liver elasticity-based imaging techniques are being developed, including ultrasound-based techniques and 3-D magnetic resonance (MR) elastography [87]. Ultrasound elastography can be currently performed by different techniques, which are

(0.5–4.4 m/sec). This limits the definitions of cut-off values for discriminating certain fibrosis stages and thus for making management decisions. Finally, quality criteria for correct interpretation of pSWE results remain to be defined.

2D-SWE is based on the combination of a radiation force induced in tissues by focused ultrasonic beams and a very high frame rate ultrasound imaging sequence capable of catching in real time the transient propagation of resulting shear waves [96]. The size of the region of interest can be chosen by the operator. 2D-SWE has also the advantage of being implemented on a commercially ultrasound machine (Aixplorer®, Supersonic Imagine, Aix en Provence, France) with results expressed either in m/sec or in kPa at a wide range of values (2–150 kPa). Its failure rate is significantly lower than that of TE [97–99], particularly in patients with ascites [98,99], but not in obese patients when the XL probe is used for TE (10.4% *vs.* 2.6%, respectively) [100]. Similar to pSWE/ARFI, quality criteria for 2D-SWE remain to be defined.

MR elastography uses a modified phase-contrast method to image the propagation characteristics of the shear wave in the liver [101]. Elasticity is quantified by MR elastography (expressed in kPa) using a formula that determines the shear modulus, which is equivalent to one-third the Young's modulus used with TE [102]. The theoretical advantages of MR elastography include its ability to analyze almost the entire liver and its good applicability in patients with obesity or ascites. However, MR elastography remains currently too costly and time-consuming to be used in routine practice and cannot be performed in livers of patients with iron overload, because of signal-to-noise limitations.

Many studies have also reported the influence of the serum ALT levels on LS and have all concluded that ALT has an effect on fibrosis beyond a correlation (Reference 2-4).

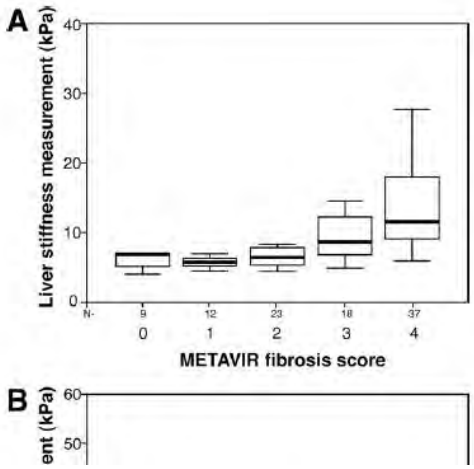
Reference2:

CLINICAL GASTROENTEROLOGY AND HEPATOLOGY 2008;6:1027–1035

Assessment of Fibrosis by Transient Elastography Compared With Liver Biopsy and Morphometry in Chronic Liver Diseases

GRACE LAI-HUNG WONG,*,‡ VINCENT WAI-SUN WONG,*,‡ PAUL CHEUNG-LUNG CHOI,§ ANTHONY WING-HUNG CHAN,§ RICHARD HOI-LEONG CHUM,‡ HENRY KAI-WING CHAN,‡ KENNETH KA-KI LAU,‡ ANGEL MEI-LING CHIM,‡ KAREN KA-LAM YIU,*,‡ FRANCIS KA-LEUNG CHAN,*,‡ JOSEPH JAO-YAO SUNG,*,‡ and HENRY LIK-YUEN CHAN*,‡

*Institute of Digestive Disease, ‡Department of Medicine and Therapeutics, and §Department of Anatomical and Cellular Pathology, Chinese University of Hong Kong, Hong Kong SAR, China



Factors Affecting Liver Fibrosis as Measured by Liver Stiffness Measurement

Higher LSM was associated with higher serum ALT level (Table 3). This indicated that a more severe hepatic inflammation was associated with higher LSM. No association between Brunt necroinflammation score and LSM could be demonstrated. Other factors including age, gender, etiology of liver disease, comorbidities (including hypertension, diabetes, dyslipidemia, overweight, and metabolic syndrome) had no effect on LSM.

Effect of Transaminase Levels on Liver Histology and Liver Stiffness Measurement

Because ALT is a commonly used parameter to reflect hepatic inflammation and approximately half of the patients had normal or elevated ALT levels, we analyzed the relationships of ALT, liver histology, and LSM. Sixty-two (47%) patients had ALT levels higher than the upper limit of normal (ULN), with similar numbers of patients with bridging fibrosis or liver cirrhosis as those with normal ALT levels. Viral hepatitis pa-

Reference 3:

JGH Journal of Gastroenterology and Hepatology

JGHF

doi:10.1111/jgh.12840

HEPATOLOGY

Transient elastography compared to serum markers to predict liver fibrosis in a cohort of Chinese patients with chronic hepatitis B

Jidong Jia,* Jinlin Hou,† Huiguo Ding,‡ Guofeng Chen,§ Qing Xie,¶ Yuming Wang,** Minde Zeng,†† Jingmin Zhao,‡‡ Tailing Wang,§§ Xiqi Hu¶¶ and D. Schuppan***,†††

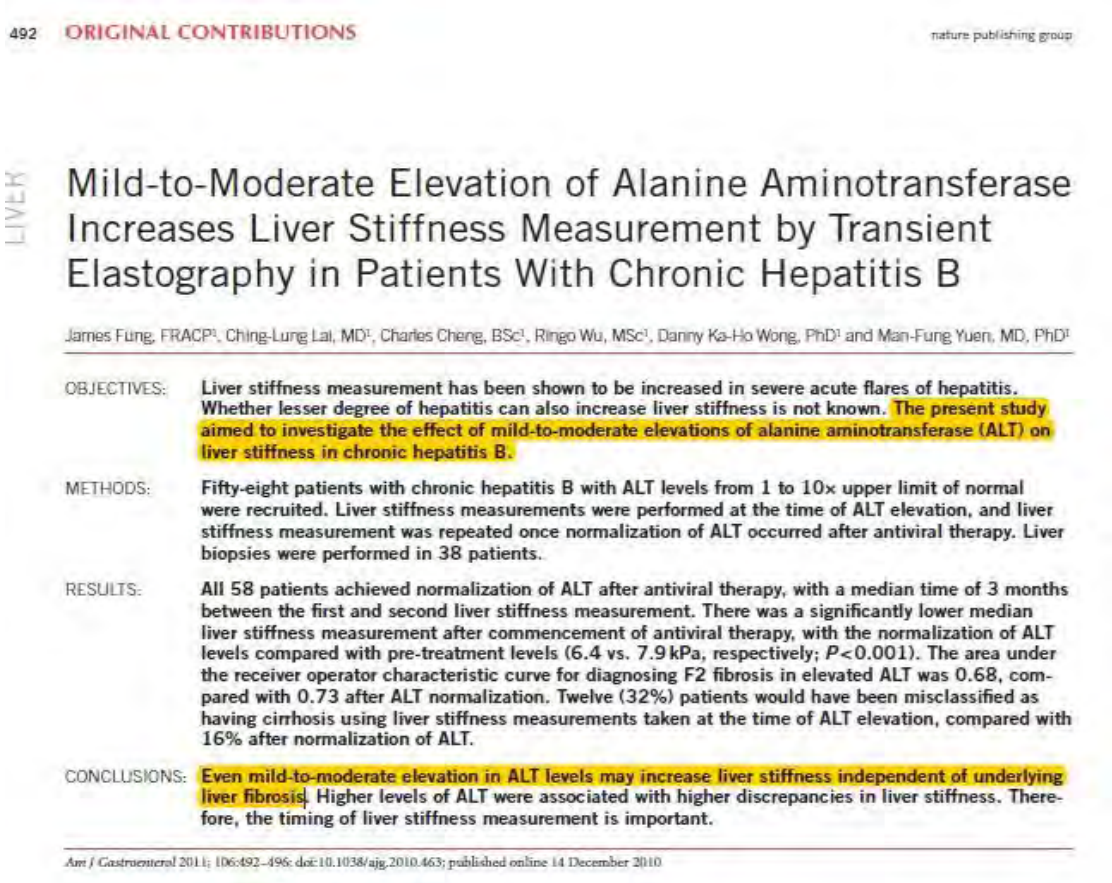
Effect of ALT levels on LSM performances and optimal cutoffs values

Effect on diagnostic performances. To assess the effect of ALT on cutoffs and diagnostic performances, we stratified the 465 patients with available LSM and ALT data into four categories: 200 (43%) had ALT below upper limit of normal (ULN), 166 (36%) between one and three times, 53 (11%) between three and five times, and 46 (10%) > 5 times the ULN. AUROCs for ≥ F2 were 0.78, 0.82, 0.77, 0.78, and 0.82 for patients with normal ALT, ALT one to three times, three to five times, and > 5 ULN, as well as all patients, respectively ($P > 0.05$). AUROCs for diagnosis of F4 tended to decrease with increase of ALT levels, with values of 0.92, 0.92, 0.90, 0.77, and 0.90 for patients with normal ALT, ALT one to three times, three to five times, and > 5 ULN, and all patients, respectively. Again, this decrease was not significant ($P > 0.05$).

Therefore, although APRI and FIB-4 had previously been shown to be useful to stage liver fibrosis in CHC patients, our results suggest that in Chinese patients with CHB both panels are significantly inferior to LSM measurement. Notably, in CHB elevated ALT levels seem to affect the diagnostic performance of these markers for intermediate and advanced stages of fibrosis.

Numerous studies have demonstrated the influence of necroinflammatory activity on LSM, with increased stiffness values being associated with elevated transaminase levels.[25] In the present study, as expected, patients with elevated ALT levels showed higher LSM values. However, performances of LSM was not significantly compromised with these ALT elevations, and predictive value remained identical for diagnosis of F ≥ 2, while there was a decrease, yet insignificant, of diagnostic performance for predicting cirrhosis. Notably, applying a specific algorithm

Reference 4:



LIVER

Mild-to-Moderate Elevation of Alanine Aminotransferase Increases Liver Stiffness Measurement by Transient Elastography in Patients With Chronic Hepatitis B

James Fung, FRACP[1], Ching-Lung Lai, MD[1], Charles Cheng, BSc[1], Ringo Wu, MSc[1], Danny Ka-Ho Wong, PhD[1] and Man-Fung Yuen, MD, PhD[1]

OBJECTIVES: Liver stiffness measurement has been shown to be increased in severe acute flares of hepatitis. Whether lesser degree of hepatitis can also increase liver stiffness is not known. The present study aimed to investigate the effect of mild-to-moderate elevations of alanine aminotransferase (ALT) on liver stiffness in chronic hepatitis B.

METHODS: Fifty-eight patients with chronic hepatitis B with ALT levels from 1 to 10× upper limit of normal were recruited. Liver stiffness measurements were performed at the time of ALT elevation, and liver stiffness measurement was repeated once normalization of ALT occurred after antiviral therapy. Liver biopsies were performed in 38 patients.

RESULTS: All 58 patients achieved normalization of ALT after antiviral therapy, with a median time of 3 months between the first and second liver stiffness measurement. There was a significantly lower median liver stiffness measurement after commencement of antiviral therapy, with the normalization of ALT levels compared with pre-treatment levels (6.4 vs. 7.9 kPa, respectively; $P<0.001$). The area under the receiver operator characteristic curve for diagnosing F2 fibrosis in elevated ALT was 0.68, compared with 0.73 after ALT normalization. Twelve (32%) patients would have been misclassified as having cirrhosis using liver stiffness measurements taken at the time of ALT elevation, compared with 16% after normalization of ALT.

CONCLUSIONS: Even mild-to-moderate elevation in ALT levels may increase liver stiffness independent of underlying liver fibrosis. Higher levels of ALT were associated with higher discrepancies in liver stiffness. Therefore, the timing of liver stiffness measurement is important.

Am J Gastroenterol 2011; 106:492–496; doi:10.1038/ajg.2010.463; published online 14 December 2010

In addition to LS, other confounding factors that could influence the non-invasive diagnosis were also detected, such as age (Reference 5).

Reference 5:

LIVER

Age as a Confounding Factor for the Accurate Non-Invasive Diagnosis of Advanced NAFLD Fibrosis

Stuart McPherson, BSc, MBChB, MD, FRCP[1,2], Tim Hardy, BSc, MBBS[1,2], Jean-Francois Dufour, MD, PhD[3], Salvatore Petta, MD, PhD[4], Manuel Romero-Gomez, MD, PhD[5], Mike Allison, BSc(Hons), MD, PhD[6], Claudia P. Oliveira, MD, PhD[7], Sven Francque, MD, PhD[8], Luc Van Gaal, MD, PhD[9], Jörn M. Schattenberg, MD, PhD[10], Dina Tiniakos, MD, PhD[1,2], Alastair Burt, BSc (Hons), MBChB, MD (Hons), FRCPath, FRCP, FRCPA, FRSB, F AcadMEd, FAHMS[11], Elisabetta Bugianesi, MD, PhD[12], Vlad Ratziu, MD, PhD[13], Christopher P. Day, MA, MB BChir, MD, PhD, FRCP, FRCPE, FMedSci[1,2] and Quentin M. Anstee, BSc, MB BS, PhD, FRCP[1,2]

OBJECTIVES: **Non-invasive fibrosis scores are widely used to identify/exclude advanced fibrosis in patients with non-alcoholic fatty liver disease (NAFLD). However, these scores were principally developed and validated in patients aged between 35 and 65 years of age. The objective of this study was to assess the effect of age on the performance of non-invasive fibrosis tests in NAFLD.**

METHODS: **Patients were recruited from European specialist hepatology clinics. The cohort was divided into five age-based groups: ≤35 (*n*=74), 36–45 (*n*=96), 46–55 (*n*=197), 56–64 (*n*=191), and ≥65 years (*n*=76), and the performance of the aspartate aminotransferase (AST)/alanine transaminase (ALT) ratio, fibrosis 4 (FIB-4), and NAFLD fibrosis score (NFS) for advanced fibrosis (stage F3–F4) for each group was assessed using liver biopsy as the standard.**

RESULTS: **Six hundred and thirty-four patients were included. The diagnostic accuracy of the AST/ALT ratio was lower than NFS and FIB-4 in all the age groups. The AST/ALT ratio, NFS, and FIB-4 score performed poorly for a diagnosis of advanced fibrosis in those aged ≤35 years (area under the receiver operating characteristic curves (AUROCs 0.52, 0.52, and 0.60, respectively). For all groups >35 years, AUROCs for advanced fibrosis were similar for the NFS and FIB-4 score (range 0.77–0.84). However, the specificity for advanced fibrosis using the FIB-4 and NFS declined with age, becoming unacceptably low in those aged ≥65 years (35% for FIB-4 and 20% for NFS). New cutoffs were derived (and validated) for those aged ≥65 years, which improved specificity to 70% without adversely affecting sensitivity (FIB-4 2.0, sensitivity 77%; NFS 0.12, sensitivity 80%).**

CONCLUSIONS: **The NFS and FIB-4 scores have similar accuracy for advanced fibrosis in patients aged >35 years. However, the specificity for advanced fibrosis is unacceptably low in patients aged ≥65 years, resulting in a high false positive rate. New thresholds for use in patients aged ≥65 years are proposed to address this issue.**

Am J Gastroenterol 2017; 112:740–751; doi:10.1038/ajg.2016.453; published online 11 October 2016

ment of "raised" transaminases (serum ALT or AST >40 IU/ml) in 464 (73%) patients and for the staging of disease in 170 (27%) patients with imaging evidence of steatosis and "normal" transaminase levels. The mean age was 51±12 years. Forty-three percent of the cohort were diabetic and the mean body mass index was 34.5±6 kg/m². In total, 461 (73%) patients had histological evidence of NASH and the median NAFLD activity score was 4 (1–8). Overall, the median fibrosis stage was F1 (F0–F4); 164 patients (25%) had advanced liver fibrosis (stage F3–F4).

Effect of age on clinical factors in patients with NAFLD

In order to assess the effect of age on clinical accuracy of simple non-invasive scores for NAFLD fibrosis, the cohort was stratified

Effects of age on specificity of AST/ALT ratio for advanced fibrosis

Overall, there was a significant negative association between age and serum ALT ($P<0.001$), whereas there was no significant relationship between age and AST. When the relationship between age and serum ALT in patients with no/mild fibrosis (stage F0–F1) or moderate-to-advanced fibrosis (stage F2–F4) were analyzed separately to correct for fibrosis, the significant negative relationship between serum ALT and age persisted ($P<0.001$ for both see **Figure 2a,b**), suggesting the age-related fall in ALT level was independent of fibrosis stage. This relationship also persisted when males and females were analyzed separately (data not

Study Highlights

WHAT IS CURRENT KNOWLEDGE

- Simple non-invasive fibrosis scores, such as the non-alcoholic fatty liver disease (NAFLD) fibrosis score, fibrosis 4 (FIB-4) score, and aspartate aminotransferase (AST)/alanine transaminase (ALT) ratio, are widely used to exclude advanced fibrosis in patients with NAFLD.
- These non-invasive scores have not been evaluated in patients at the extremes of age (<35 years or >65 years).

WHAT IS NEW HERE

- The NAFLD fibrosis score, FIB-4 score, and AST/ALT ratio perform poorly in patients aged <35 years.
- The NAFLD fibrosis score and FIB-4 have low specificity for advanced fibrosis in patients aged >65 years leading to a high false positive rate.
- New cutoffs for excluding advanced fibrosis for patients aged >65 years have been derived (and validated) for the NAFLD fibrosis score and FIB-4 score, which reduced the false positive rate without adversely affecting sensitivity.

As you can see, studies have concluded the effect of ALT, not correlation.

2. Responses to comment (In the last paragraph of the result section, what did the authors mean “non-patented tests”?)

Responses:

According to the Asian-Pacific Association for the Study of the Liver (APASL) consensus guidelines for the invasive and non-invasive assessment of hepatic fibrosis, serum markers for the diagnosis of liver fibrosis can be divided into non-patented biomarkers and patented biomarkers. Most of the non-patented biomarkers are derived from routine laboratory tests, which are the basic components of the non-invasive tests. The most commonly used non-invasive tests are the (AST)-to-platelet (PLT) ratio index (APRI), FIB-4, King’s score, Forns index and gamma-glutamyl transpeptidase (GGT)-to-platelet (PLT) ratio (GPR), which all belong to the non-patented tests(Reference 6,7). In order to describe this problem clearly and uniformly, we changed “non-patented” into “non-invasive”.

Reference 6:

Hepatol Int (2017) 11:1–30
DOI 10.1007/s12072-016-9760-3

GUIDELINES

Asian-Pacific Association for the Study of the Liver (APASL) consensus guidelines on invasive and non-invasive assessment of hepatic fibrosis: a 2016 update

Gamal Shiha[1,2] · Alaa Ibrahim[3] · Ahmed Helmy[6] · Shiv Kumar Sarin[4] · Masao Omata[5] · Ashish Kumar[7] · David Bernstien[8] · Hitushi Maruyama[9] · Vivek Saraswat[10] · Yogesh Chawla[11] · Saeed Hamid[12] · Zaigham Abbas[13] · Pierre Bedossa[14] · Puja Sakhuja[15] · Mamun Elmahatab[16] · Seng Gee Lim[17] · Laurentius Lesmana[18] · Jose Sollano[19] · Ji-Dong Jia[20] · Bahaa Abbas[21] · Ashraf Omar[22] · Barjesh Sharma[23] · Diana Payawal[24] · Ahmed Abdallah[25] · Abdelhamid Serwah[26] · Abdelkhalek Hamed[27] · Aly Elsayed[28] · Amany AbdelMaqsod[29] · Tarek Hassanein[30] · Ahmed Ihab[31] · Hamsik GHaziuan[32] · Nizar Zein[33] · Manoj Kumar[4]

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surrogate marker, i.e., changes in these biomarkers can predict the clinical outcome (efficacy—E).
3. Diagnostic biomarkers enabling identification of patients within the population and identification of subgroups within the diseased population (diagnostic—D).
4. Biomarkers that identify subjects with high risk of progression (prognostic—P)
5. Biomarkers not fully validated in a study population and therefore can be used solely for scientific investigations (investigatory—I).

Classification by the BIPED system may enable researchers from different backgrounds to communicate in a robust assessment framework, with a special focus on diagnostic, prognostic and possible burden of disease evaluations [77].

Serum markers for diagnosis of liver fibrosis

Class I biomarkers

Individual tests in general are too insensitive to be used, and hence class I biomarkers are often tested in panels. Only two test panels that underwent significant clinical evaluation. The first is MP3, which comprises amino-terminal propeptide of procollagen type III (PIIINP), a marker of fibrogenesis, and MMP-1. However, few studies have utilized this MP3 test and are limited to patients with chronic HCV infection [78]. The second is the enhanced

Class II biomarkers

These can be divided into simple panels, which are derived from routine laboratory tests or patented panels, which are commercial kits of a large panel of tests. Meta-analyses of studies were limited, and a search found two studies [83, 84]. Poynard et al. examined biomarkers of liver fibrosis, and his main objective was to examine the diagnostic utility of the biomarker for advanced fibrosis defined as ≥F2 (METAVIR system) based entirely on the AUROC curve [83]. The study also adjusted for spectrum bias by using DANA analysis. There were 62 quality measures, and validation was based on the number of studies and number of patients. Among 2237 studies, 14 biomarkers were found to be validated, 9 were not patented, and 5 were patented (Tables 7 and 8). FibroTest (FT) was the most studied test with 33 different populations including 6549 patients and 925 controls. The mean diagnostic value for the diagnosis of advanced fibrosis (≥F3) assessed using AUROC curves was 0.84 [95 % confidence interval (CI), 0.83–0.86], without significant difference between the causes of CLD, hepatitis C, hepatitis B, alcoholic or NAFLD. High-risk profiles of false-negative/-positive of FT were present in 3 % of populations (mainly those with Gilbert syndrome, hemolysis and acute inflammation). FT had higher accuracy than the most used nonpatented test, APRI. No significant difference has been observed among the five patented tests [83].

Table 7 List of biomarkers from Poynard et al.'s meta-analysis [83]

Non-patented biomarkers		Patented biomarkers	
PGA index	PI, GGT, ApoA1	FT/FS (Fibrotest/fibrosure)	A2M, haptoglobin, ApoA1, bilirubin, GGT, age, gender
AP index (age-platelets)	Plt, age	FSP (Fibrospect II)	A2M, HA, TIMP-1
Bonacini index	Plt, ALT, AST	ELF (enhanced liver fibrosis)	HA, PIIINP, TIMP-1
Pohl score	Plt, AST	FM (Fibrometer)	Plt, AST, A2M, HA, PT, age, gender
Forns index	Plt, cholesterol, age	HS (Hepascore)	A2M, HA, GGT, age, gender
APRI	Plt, AST		
MP3 index	PIIINP, MMP1		
FIB-4 (Fibrosis-4)	Plt, AST, ALT, age		
Fibroindex	Plt, AST, γ-globulins		

ApoA1 apolipoprotein A1, *A2M* alpha-2 macroglobulin, *ALT* alanine transaminase, *AST* aspartate transaminase, *APRI* AST/platelet ratio index, *GGT* gamma glutamyle transferase, *HA* hyaluronic acid, *MMPs* matrix metalloproteinase, *PIIINP* amino-terminal propeptide of procollagen type III, *Plt* platelets, *PT* prothrombin time

Reference 7:

Clin Res Hepatol Gastroenterol. 2014 Sep;38(4):432-9. doi: 10.1016/j.clinre.2014.04.006. Epub 2014 Jun 9.

Variability in definitions of transaminase upper limit of the normal impacts the APRI performance as a biomarker of fibrosis in patients with chronic hepatitis C: "APRI c'est fini ?".

Perazzo H[1], Pais R[2], Munteanu M[3], Ngo Y[3], Monneret D[4], Imbert-Bismut F[4], Moussalli J[1], Lebray P[1], Benhamou Y[1], Thabut D[1], Ratziu V[1], de Ledhingen V[5], Poynard T[6]; FibroFrance Group; EPIC3 Group.

⊕ Author information

Abstract

BACKGROUND: The aspartate aminotransferase platelet ratio index (APRI) is a validated non-patented blood test for diagnosing fibrosis or cirrhosis in patients with chronic hepatitis C. We assess the impact of two limitations, the variability of the upper limit of normal for aspartate aminotransferase (AST-ULN) and the risk of overestimating fibrosis stage due to necroinflammatory activity.

METHODS: The variability of AST-ULN was assessed by an overview of the literature and an assessment of AST-ULN in 2 control populations 7521 healthy volunteers and 393 blood donors. We assessed the impact of AST-ULN variability on APRI performance for estimating fibrosis prevalence and on the Obuchowski measure using individual data of 1651 patients with APRI, FibroTest and biopsy.

RESULTS: The overview, and the analysis of the control populations found that ULN-AST ranged from 26 to 49 IU/L according to gender, body mass index and serum cholesterol. When this AST-ULN variability was applied to the chronic hepatitis group, the prevalence of advanced fibrosis and cirrhosis as presumed by APRI varied (P<0.001) from 34.7% to 68.5%, and from 11.4% to 32.3%, respectively. This spectrum effect induced variability in APRI performance, which could be similar 0.862 (if AST-ULN=26 IU/L) or lower 0.820 (AST-ULN≥30IU/L) than the stable FibroTest performance (0.867; P=0.35 and P<0.0001 respectively). When applied to 18 acute hepatitis C patients, the rate of false positives of APRI varied from 0% to 61% due to AST-ULN.

CONCLUSION: The AST-ULN variability is high highly associated with the variability of metabolic risk factors between the different control groups. This variability induces a spectrum effect, which could cause misleading interpretations of APRI performance for the staging of fibrosis, comparisons of APRI with other non-invasive tests, and estimates of false positive rate.

PMID: 24924901 DOI: 10.1016/j.clinre.2014.04.006

3. Responses to comment (Some language editing is needed)

Responses:

We apologize for our poor English language usage. The manuscript was resubmitted to an editing service for a thorough review of the language. The amendments are highlighted in cyan (format for manuscript) in the revised manuscript. For example, in the discussion section, “we are the

first study." was changed into "this study is the first".

To Reviewer 2 (Reviewer's code: 02860897):

Responses to comment (In the present study, object was limited to cases due to HBV. Considering the characteristics of formula, can we apply these formula regardless of underlying disease?)

Responses:

First, chronic hepatitis B virus (HBV) infection is a major public health issue worldwide and is also the most common chronic viral infection in China. Second, most non-invasive fibrosis tests have been developed primarily for chronic hepatitis patients; their use in assessing liver fibrosis in CHB patients remains controversial. For example, the Forns index and APRI were developed for chronic hepatitis C patients. FIB-4 was developed for HIV-HCV patients. Thus, the aim of this study was to explore the effect of alanine aminotransferase (ALT) on the performance of non-invasive fibrosis tests in chronic hepatitis B (CHB) patients.

Special thanks to you for your valuable comments. The effect of alanine aminotransferase (ALT) on the performance of non-invasive fibrosis tests in other chronic liver disease will be evaluated in the future.

To Reviewer 3 (Reviewer's code: 00069423):

Responses to comment (The controversy as to the applicability of

currently used non-invasive fibrosis test for patients with hepatitis B is again brought to light by the authors of this paper. While non-invasive fibrosis tests were originally generated and applied effectively to patients with chronic hepatitis B. other studies have shown conflicting results in the past. In this manuscript, the authors highlighted the influence of the ALT levels on the accuracy of non-invasive fibrosis tests and indicated the best usage of these tests was for CHB patients with normal ALT. The discussion is excellent in support of their finding. I believe these findings are significant and should be considered when using the non-invasive fibrosis tests for patients with CHB. Further studies may be able to add further applicability of the much needed non-invasive tests in the future. There are a few typos and grammatical errors).

Responses:

We apologize for our poor writing and grammatical errors. As the Reviewer noted, the manuscript does contain a few typos and grammatical errors. We have revised the manuscript carefully according to the Reviewer's suggestion. We also resubmitted our manuscript to an editing service to further polish the language. The amendments are highlighted in cyan (format for manuscript) in the revised manuscript.

We tried our best to improve the manuscript and have made some revisions. These changes do not influence the content or framework of the

paper. We did not list the changes here but instead highlighted them in yellow (the editor's and reviewer's comments/suggestions) and cyan (language editing and polishing) in the revised paper. We very much appreciate the editor's and reviewers' earnest efforts and hope that the corrections will meet with approval. Once again, thank you very much for your comments and suggestions.

Best Regards,

Lin Wang