ANSWERING REVIEWERS

March 07, 2014

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 8807-Review).

Title: Real time shear wave elastography in chronic liver disease: Accuracy for predicting liver fibrosis in comparison with serum markers

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The manuscript has been improved according to the suggestions of reviewers:
1. Format has been updated

2. Revision has been made according to the suggestions of the reviewer
   Please see the point-by-point answers to the reviewers' comments below.

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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Point-by-point Answers to the reviewers' comments:

A) Reviewed by 01490498

This is a retrospective study assessing the utility of SWE is a heterogenous group of patients with regard to aetiology of liver disease. This is in contrast to the two other major studies which only assessed patients with viral hepatitis. The use of serum markers is interesting. The small numbers is a limitation.
I have some comments:

1. Can the authors confirm that all patients were fasted prior to the assessment of SWE?

Answer) Sampling for serum markers, SWE, and liver biopsy were performed in the overnight fasting state in the morning of the same day during admission. We already described this point in the section of methods and materials.

2. Give that the technique does not appear to perform any better than HA and Type IV collages, what is the justification for using this technique?

Answer) As you know, to evaluate liver fibrosis besides liver biopsy, there are 2 kinds of alternative non-invasive techniques such as various serum biomarkers of fibrosis and liver stiffness measurement (LSM). For the diagnosis of significant fibrosis, performances of transient elastography (TE) as a prototype of LSM and serum biomarkers have been shown to be equivalent in patients with chronic viral hepatitis. However, these two methodologies are not perfect and each has some limitations. One limitation of biomarkers is that none is liver-specific and they may be influenced by changes in their clearance and excretion. For instance, increased levels of hyaluronic acid occur in the post-prandial state or in aged patients with chronic inflammatory processes such as rheumatoid arthritis. Transient elastography, as a prototype of LSM, also has some limitations such as its limited accessibility and reproducibility and direct influence of liver stiffness by various conditions of the liver like edema, congestion and inflammation except liver fibrosis itself. To overcome these limitations of both methods and to improve the diagnostic performance for significant fibrosis and cirrhosis, combinations of tests have been considered.

As you comment, although SWE does not appear to perform any better than HA and Type IV collages evaluated in this study, two alternative methods of blood tests and elastography can be complementary. Especially, transient elastography has been extensively studied but more recently introduced real-time shear wave elastography has been much less studied and need accumulation of more data. This study can add data. However, to demonstrate the justification for 2D-SWE for evaluation of liver fibrosis, large prospective studies are needed.

3. I am somewhat surprised by the inferior diagnostic performance in patients with advanced fibrosis and in particular cirrhosis. This is worse that in other studies. Would the authors like to comment?

Answer) As you comment, most other studies showed that TE appears as the most accurate method for the diagnosis of cirrhosis in patients with viral hepatitis when compared with currently available biomarkers and routine blood tests. In our study, SWE showed similar, not inferior nor worse, accuracy to diagnose cirrhosis compared to HA and type IV collagen as shown in table 4 (AUCs of SWE, HA and type IV collagen : 0.877, 0.879, 0.850, respectively). We cannot explain this difference exactly at this point, but one possible explanation is the heterogeneity and small numbers of the study population. Most reports carried out the study about TE targeting patients with chronic hepatitis C and less frequently with chronic hepatitis B. However, patients with CHC and CHB occupied only about 57% in our study (26% and 33%, respectively). Notably, AUC (0.914) of SWE for diagnosis of advanced fibrosis was higher than AUCs (0.819, 0.793) of HA and type IV collagen in subgroup analysis of chronic viral hepatitis patients as shown in supplementary table 1 (table S1). So, this possibility may be supported by the result. In the near future, it should be further evaluated by large prospective

studies.

According to your comments (2 and 3), we revised our manuscript in discussion section as follows: ~~ Especially, most other studies showed that TE appears as the most accurate method for the diagnosis of cirrhosis in patients with viral hepatitis when compared with currently available biomarkers and routine blood tests. However, in our study SWE does not appear to perform significantly better than HA and Type IV collages evaluated. Furthermore, our results that SWE had only similar, but not inferior nor worse, accuracy to diagnose cirrhosis compared to HA and type IV collagen as shown in table 4 (AUCs of SWE, HA and type IV collagen: 0.877, 0.879, 0.850, respectively). We cannot explain this difference exactly at this point, but one possible explanation is the heterogeneity and small number of the study population. Most reports carried out the study about TE targeting patients with chronic hepatitis C and less frequently with chronic hepatitis B. However, patients with CHC and CHB occupied only about 57% in our study (26% and 33%, respectively). Notably, AUC (0.914) of SWE for diagnosis of advanced fibrosis was higher than AUCs (0.819, 0.793) of HA and type IV collagen in subgroup analysis of chronic viral hepatitis patients as shown in supplementary table 1 (table S1). So, this possibility may be supported by this result. On the other hand, although aforementioned studies reported that TE was superior to serum markers in detecting hepatic fibrosis in hepatitis C patients[8, 11, 23, 26], a recent review article described that the performances of TE and serum biomarkers have been shown to be equivalent for the diagnosis of significant fibrosis in patients with chronic viral hepatitis[21]. Therefore, this issue should be further evaluated by large prospective studies in the near future.

Meanwhile, the two methodologies of biomarkers and elastography are not perfect and each has some limitations. One limitation of biomarkers is that none is liver-specific and they may be influenced by changes in their clearance and excretion. For instance, increased levels of hyaluronic acid occur in the post-prandial state or in aged patients with chronic inflammatory processes such as rheumatoid arthritis[21]. Elastography also has some limitations such as its limited accessibility and reproducibility and direct influence of liver stiffness by various conditions of the liver like edema, congestion and inflammation except liver fibrosis itself. On the other hand, the two alternative methods of biomarkers and elastography can be complementary. Therefore, combinations of both methods can be considered to overcome the limitations and improve the diagnostic performance for significant fibrosis and cirrhosis. For this, future large prospective studies are necessary.

4. There are several errors in grammar and spelling. I suggest the entire article is carefully proof read and corrected by an appropriate service.

Answer) We are sorry for your inconvenience and thank you for your point-outs. We carefully reviewed and corrected errors in grammar and spelling again.

B) Reviewed by 0058401

Congratulations for the work. I made osomeberatoc restrictions that do not intrefer in the quality of the work.

Answer) Thank you for your congratulations in advance.

C) Reviewed by 00071220

I had the opportunity to review a paper “Real time shear wave elastography in chronic liver diseases: Accuracy for predicting liver fibrosis, in comparison with serum markers”, and I found very interesting. There is no problem to publish the manuscript.

Answer) Thank you for your positive review.

D) Reviewed by 00542353

In this study authors evaluated the correlation between liver stiffness measurement (LSM) by real-time shear wave elastography (SWE) and liver fibrosis stage. They enrolled 70 consecutive patients with various chronic liver

diseases (HBV, HCV, non-alcoholic liver disease and other diseases including autoimmune hepatitis and unknown cause). The major result is that LSM by SWE showed a significant correlation with the severity of liver fibrosis, maximally identifying more advanced degrees of disease. The non-invasive assessment of liver fibrosis is a really interesting field of research, however, some limitations of the present study should be addressed. ?

1) The first concern is the lack of originality. Similar studies have been already published about this issue, sometimes with larger study populations. ?

Answer) As you comment, several other studies have been published recently. However, studies for liver fibrosis staging using 2D-SWE are still limited although TE has been extensively studied. In addition, these studies mostly targeted patients with chronic hepatitis C. In our study, the various causes of chronic liver diseases are included and viral causes occupied only about 57%. Especially, our study was conducted in comparison with serum biomarkers related with liver fibrosis, different from other studies.

2) The extreme heterogeneity of the population is not a point of strength, but a source of variability. A higher sample size could be necessary to allow for subgroup analysis. A sample size assessment could be useful to address this issue. ?

Answer) Your point is correct. This point is a main weakness of this study. Our study only showed preliminary results. Sample size should be increased to assess the influence of the cause of liver disease on the variability of LSM by SWE. We hope so in a large prospective study in the near future. We already described this weak point of this study in the discussion.

3) Some studies showed that transient elastography and acoustic-radiation-force impulse elastography have a higher accuracy for the evaluation of fibrosis as compared with real-time elastography. This should be discussed by authors.

Answer) Thank you for your detailed comments.

To our regret, we did not compare SWE to other US elastography techniques such as TE and ARFI. However, the theoretical advantage of SWE is that it provides information on shear wave speed in a 2-D area of varing centimeters and not in point or line as do ARFI or TE. In addition, some recent studies showed SWE had similar or higher accuracy for the evaluation of fibrosis as compared with TE and ARFI, different results from your points as follows: 1) The first clinical study using SWE published by Bavu et al (in our reference 16) in a cohort of 113 patients with histologically proven chronic HCV showed that the AUROCs for SWE were better than those from TE performed in the same session for F≥2, F≥3 and F4 (AUROCs of 0.95 vs 0.85 for F ≥ 2, 0.96 vs 0.86for F ≥ 3 and 0.97 vs 0.94 for F = 4, respectively). 2) Ferraioli et al [in our reference 17] enrolled 121 patients with chronic hepatitis C who underwent SWE, TE and liver biopsy on the same day and compared SWE with TE. The results showed that there was no significant difference between the AUROCs of TE and SWE for severe fibrosis (0.96 vs 0.98) and cirrhosis (0.96 vs 0.98). 3) A more recent study compared the feasibility of three elastographic methods of TE, ARFI and SWE. Reliable measurements were obtained in a significantly higher percentage by means of ARFI as compared with TE and SWE: 92.1% vs.72.2% ($p<0.0001$) and 92.1% vs. 71.3% ($p<0.0001$). In subjects in whom reliable LS measurements were obtained by all three elastographic methods, the accuracy was similar for ARFI and SWE for diagnosing significant fibrosis and cirrhosis, considering TE as reference method. [Acoustic radiation force impulse and supersonic shear imaging versus transient elastography for liver fibrosis assessment. Ultrasound Med Biol 2013; 39: 1933-1941.]

We revised the discussion section in the manuscript according to your comments as follows:

To evaluate liver fibrosis besides liver biopsy, there are 2 kinds of alternative non-invasive techniques: 1) biological approach such as various serum biomarkers of fibrosis and 2) physical approach of imaging tests such

as LSM using ultrasonography or magnetic resonance[21]. And there are 3 kinds of shear wave elastography available to evaluate liver fibrosis by LSM at present: a) TE (FibroScan), as a prototype of LSM, which has been extensively studied and accepted a very valuable method; b) "Point" shear waves elastography– ARFI quantification (Siemens, Phillips); c) real-time (2 dimensional) shear waves elastography (2D-SWE, Supersonic Imagine, Aixplorer system), which is most recently introduced[22]. The theoretical advantage of SWE is that it provides information on shear wave speed in a 2-D area of varying centimeters and not in point or line as do ARFI or TE. Although some recent studies showed 2-D SWE had similar or higher accuracy for the evaluation of fibrosis as compared with TE and ARFI, they are still limited and mostly targeted patients with hepatitis C. Additional data are necessary especially on other disease etiologies, and more information such as comparison studies with other elastographic techniques such as TE and ARFI is needed for the introduction of this method in clinical practice. Therefore, in this study, ~~

E) Reviewed by 01550345

Some histological data of the patients' liver would help to improve the paper.

Answer) Thank you for your comment. **We added a figure** of a representative case of a chronic hepatitis B patient with histology and LSM by SWE as an example **(Figure 2).**

In the result section of the manuscript, we added a sentence that described "Figure 2 showed an example of (A) histologic findings of advanced liver fibrosis (F3) on liver biopsy in a patient with chronic hepatitis B and (B) LS value of 13.0 kPa measured by SWE on the same day, suggesting advanced liver fibrosis." And we added a "Figure 2. A representative case of chronic hepatitis B patient demonstrating liver histology and liver stiffness measurement by real-time shear wave elastography: (A) microscopic finding of liver biopsy showed dense septal (F3) and portal fibrosis with moderate portal and periportal inflammation (Left; H&E stain, x 40, Right: Masson Trichrome stain, x 40); (B) shear wave elastography showing liver stiffness value of 13.0 kPa, which suggested advanced liver fibrosis (F3 stage)".