

Reviewer #1

I have reviewed this interesting papers and would like to have a few comments. The data in this papers are the result of the combination of the test population (n = 108) combined with the validation group (n = 71), which was published in the PLOS ONE in June 2013 from the same group of authors. In that paper the authors showed that the composited score called Angioscore based on Ang2, age, INR, AST, platelet and GGT. The Angioscore showed better performance than all other scores. The authors also showed the level and the AUC curve graphs of Ang1, Ang2 and Ang2/Ang1 levels in F1, F2, F3, which showed promising results (PLOS ONE 2013;8:6: e66143). In term of this paper, it is the copy of the same data that combine both populations and stresses on the Ang2/Ang1 utilization. To make it better and not a copycat of the old paper, I would like to request some more detail as following.

1. The graphs showed the levels of Ang1, Ang2, Ang2/Ang1 ratio with the fibrotic stages and the statistic significant.

We thank the Reviewer #1 for suggesting us this important idea. We now include these data in the new Figure 1, which clearly show the close relationship between Angiopoietins' levels and their ratio with the stage of fibrosis in those patients. Indeed, the Ang2/Ang1 ratio gradually rises as liver fibrosis progresses. Accordingly, the respective explanation has been corrected in the main text as follows: *"Interestingly, the concentration of Ang1 decreased progressively in relation to the stage of liver fibrosis whereas Ang2 levels showed the opposite tendency (Figure 1). Furthermore, the concentration of Ang1 in the serum of cirrhotic patients was significantly lower when compared to the non-cirrhotic groups ($p < 0.001$); on the contrary Ang2 serum levels were considerably higher in patients with cirrhosis ($p < 0.01$, Figure 1). Hence, differences among fibrosis stages were more evident for Ang2/Ang1 ratio, which was further able to significantly discriminate $F > 1$."*

2. The number of F1 and F2 patients is 108 or 60.3% of the total case. Are these statistic significant come from the different between the mild (F1 and F2) and the F4, but not from the F3 (n= 40)?

We really appreciate this interesting comment. As the reviewer can perceive in the novel Figure 1 as suggested, there is a notable statistic difference between F3 and F4 ($p < 0.01$); hence, previous described differences do not seem to come from the other stages of fibrosis (F1 and F2).

3. Both Angioscore or the Ang2/Ang1 ratio are the outstanding scores and will be of useful in clinical practices. It would be better If they could show more results on the differentiation between F3 and F4, and also between the mild fibrosis (F1, F2) and moderate to severe fibrosis (F3, F4). As we always know that the risk of HCC in F3 and F4 is up to 40-60% within 10 years (or 4-6% per year). So if the score aimed for differentiate mild to moderate (F1-3) from severe F4, we will miss the F3 patients who also have high risk of HCC.

As reviewer proposed, the ability of Ang2/Ang1 ratio for discriminating $F > 1$ and $F > 2$ are now more evident in the novel Figure 1. Such important concerns have been described in the main text as explained above.

Reviewer #2

This is a study regarding the role of Ang2/Ang1 ratio as a non-invasive biomarker of fibrosis in chronic hepatitis C. Overall, the manuscript was well-written and all tables and figures were appropriate. The main findings about Ang2/Ang1 ratio was quite novel.

1. However the usefulness of it in this field was still questionable to me because there are several non-invasive tests for fibrosis in hepatitis C, such as serum Fibrotest and liver stiffness (Fibroscan), are more established and now widely available in the market.

We thank the positive evaluation of Reviewer #2 and very much appreciate the opportunity to discuss this interesting question. Discordance between liver fibrosis estimated by Transient Elastography (Fibroscan) and biopsy has been described for an important part of patients (higher BMI, older age, cholestasis... PMID: 20807336). In addition, the effectiveness of Fibroscan for distinguishing mild fibrosis (F0-2 vs. F3-4) is quite limited (6). Hence, clinicians are aware to consider alternative procedures for fibrosis staging including biomarkers and possibly biopsy, mainly in patients showing above characteristics. In this regard, in accordance with your interesting appreciations and those from the other referees, we now have noticed the outstanding correlation of Ang2/Ang1 with fibrosis and its significant ability to discriminate between low, mild and severe fibrosis as now can be observed in the new figure 1. Therefore, these remarkable data highlight the potential of these factors as a valuable option for the non-invasive assessing of liver fibrosis in different clinical settings, particularly in some of which Fibroscan has slight worth, and stimulate their further validation in higher cohorts of patients. Moreover, if so, these factors seem to be cheaper and easier to obtain than the expensive patented FibroTest, which is based on the measurement of many other compounds in a more complex and costly fashion.

2. In the discussion part, I am not sure that the sentence “Ang2/Ang1 ratio displays similar or superior precision than other proposed biomarkers such as α -Fetoprotein, 13C-ABT, HA and CK18, or tests (PAPAS, APRI, FIB4, GUCI, etc...)” is appropriate since there was no direct comparative study in the cited references. To increase the strength of this manuscript, especially this claiming, I would suggest the authors to compare the ROC of Ang2/Ang1 ratio in predicting cirrhosis with the ROCs of other non-invasive serum markers such as AST/ALT ratio, APRI, and/or FIB-4 index which are not difficult to calculate by using simple lab parameters.

We completely agree with the reviewer and apologize for this inappropriate sentence. As requested, we have performed and included the proposed comparisons and realizing that Ang2/Ang1 ratio performs similar or better than other described indices (new table 4). Thus, the respective text has been accordingly amended in the revised manuscript as follows in Material and methods section: *“Statistic differences among different cirrhosis indices were calculated by De Long test”*, Results: *“Finally, the efficacy of Ang2/Ang1 for cirrhosis staging was compared with other previously described non-invasive serum markers (AAR, APRI, FIB-4, FI and FCI). As Table 4 shows, angiopoietins ratio performs better than AAR ($p=0.01$) and similar to the other indices ($p>0.05$)”*, as well as in Discussion: *“In addition, it must be pointed out that Ang2/Ang1 ratio displays similar or superior precision than other proposed tests (AAR, APRI, FIB4, FI, and FCI.”*

Reviewer #3

The idea of assessment of Angiopoietin2/angiopoietin1 ratio as a noninvasive biomarker in cirrhosis in chronic hepatitis C is quite interesting, however, I have some comments:

1. out of 179 patients included, only 31 had liver cirrhosis on biopsy (F4) Metavir, and the final conclusion was based on a comparison between a group of 31 patients (F4 fibrosis) and another much bigger group of 148 patients (F1, F2, and F3, all together).

We really appreciate the interesting question raised up by the Reviewer. As pointed out by the referee, prevalence of the considered disease (i. e. cirrhosis) is one of most important factor that can influence the performance of a test. Therefore, clinicians' decisions must be based on studies that most closely match the genuine clinical situation. In order to avoid possible selection bias, adjusting by prevalence is highly recommended whenever the study population be quite different from that of the real world (PMID:18778913, PMID: 18765409 PMCID: PMC2917255). In this regard, we firstly would like to note that the criteria used to recruit and enroll patients in our study was not forced by the investigators, samples were employed as they were available. Amazingly, the prevalence of cirrhosis in our cohort (17.3%) was quite similar to the recently referred for CHC patients (PMID:21184757). Even so, results were further adjusted by the described prevalence (18.5) using MedCalc software.

2- The data did not show the stratification of this biomarker according to the stage of fibrosis, F1, F2, F3 and F4, was this ratio higher in F2 compared to F1?

Following Reviewers' indications, we have included the graphs showing the levels of Ang1, Ang2, Ang2/Ang1 ratio corresponding to each fibrotic stage and the respective statistic differences among them (Figure 1). As the reviewer suspected, Ang2/Ang1 ratio is higher in F2 compared to F1 as well as in F3 compared to F2.

3- Among 179 patients who had a liver biopsy, why non of them had F0 fibrosis?, was it an exclusion criterion?

We would like to apologize for including F0 patients (n=3 in our cohort) in the F1 group without the corresponding clarification. Now, we have accordingly exposed in the Material and methods section of the revised manuscript: *"Liver fibrosis was staged as F0 to F4 according to the METAVIR classification system[36]. In order to simplify, 3 patients with F0 were included in the F1 group"*.

4- Did any of these patients receive antiviral therapy?

Most patients were going to receive antiviral therapy but samples were always undertaken at baseline.