Reviewer's report

**Title:** The sweet and sour of serological glycoprotein tumor biomarker quantification

**Version:** 3  **Date:** 2 October 2012

**Reviewer:** Hisashi Narimatsu

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Comment to the author

This manuscript describes development of the CLIA method system for detection of Y-box binding protein-1 (YB-1). The authors developed a monoclonal antibody against YB-1, and determined the epitope domain of YB-1 for establishment of the CLIA system method. Moreover, the authors quantified serum YB-1 using the CLIA system method in healthy volunteers and HBV hepatitis B patients, HBV cirrhosis HBV-related cirrhotic patients, and hepatocellular carcinoma (HCC) patients for evaluation of the diagnostic rate performance of YB-1 by comparative analysis between HCC and non-HCC groups consisted of these subjects. It is described that the diagnostic performance rate of YB-1 was superior over AFP in sensitivity and accuracy, and that the combination use of YB-1 and AFP increases sensitivity and accuracy despite but decreases specificity decrease. The authors conclude that YB-1 is a potential diagnostic marker for HCC. However, it should be pointed out that further amendment of the manuscript is necessary to reach a potential level for publication in Clinica Chimica Acta. The following are some suggestions.

**Major comments**

1. As the authors described in Introduction, it has been already known that the serum level of YB-1/p18 serves as an HCC marker. Development of the new detection system and measurements with this system are the progress in this study; however, usefulness of this molecule has been reported thoroughly and is not a novel discovery. Hence, the most important point of this manuscript should be improvement of the detection accuracy of YB-1 achieved by using the CLIA system method developed by the authors. However, clear evidence of the improvement is not presented indicated in this manuscript. At least the authors should state the advantage of the developed antibody over commercially available antibodies regarding detection accuracy. Taken together, the manuscript should concentrate on the description for establishment of the new detection system method, and be recommended for a journal specialized in other than the clinical field.

2. There are numerous concerns with the experimental data and design as well as the experimental controls. Especially, as there is no clearly compiled basic clinical information provided, overall clinical verification of this study has not been secured. Furthermore, the method of statistical analyses, such as receiver
operating characteristic (ROC) analysis, decision for the cut off values, and interpretation and definition for the meaning of the combinatorial analysis are not clear. Specifically, (1) information on etiology and state of background liver disease in HCC patients should be described in Table 1. (2) Given that no discussion made on the characteristics of HCC and YB-1 values, characteristics of HCC shown in Table 1 are not necessary. (3) Regression process on for the combination of YB-1 and AFP shown in Table 5 should be clarified. (4) Table 5 should be represented by the area under the ROC curve (AUC) of the ROC analysis, not by diagnostic accuracy.

3. It is concerned that there is no strong correlation between the theme of this manuscript and Fig. 1 to Fig. 4. Although the most part of this manuscript refers to these figures, the contention of the manuscript is supposed to be the specificity of the developed antibody against a certain epitope of YB-1 peptide, which can be described sufficiently by Fig. 4 only. However, there is a serious lack of information in this Fig. 4: there is no description for the truncated forms of the YB-1 protein being expressed.

being expressed at the all protein levels.

Minor comments

1. Should provide the numerical data and units for X and Y axes in Fig 5A.
2. Significance for presenting of Fig. 6A and 6B is not clear.
3. There is no ROC curve for combination of AFP and YB-1 in Fig. 810.
4. There is no interpretation for combination of correlation coefficient and 2D plotting of AFP and YB-1.
5. Overall quality of figures, especially Fig 1, 2, 3, 4 and 5 and tables should be improved. Recommend reconsideration on appearance, lettering, and numbering and fonts can be uniformed and appearance be reconsidered for clarity.

Quality of written English: Needs some language corrections before being published

Statistical review: No, the manuscript does not need to be seen by a statistician.

Declaration of competing interests:

I declare no competing interests.