

Dear Peer reviewers,

We have made some minor revision of the language according to your suggestions, as follows:

1. **AIM:** To determine the prevalence and diagnostic value of autoantibodies in  $\alpha$ -fetoprotein (AFP)-negative hepatocellular carcinoma.

**Revision:**

**AIM:** To determine the prevalence and diagnostic value of autoantibodies in  $\alpha$ -fetoprotein (AFP)-negative hepatocellular carcinoma (HCC).

2. **RESULTS:** .....When the three autoantibodies were combined, the sensitivity reached 30.4% and the specificity remained 91.6%.

**Revision:**

**RESULTS:** .....When the three autoantibodies were combined, the sensitivity reached 30.4% and the specificity reached 91.6%.

3. *Recombinant proteins and antibodies*

..... Addgene (75 Sidney Street, Suite 550A Cambridge, MA 02139 USA)

**Revision:**

.....Addgene (Cambridge, MA, USA)

4. *Immunohistochemistry with tissue array slides*

.....DAB Substrate Kit (Beijing Zhong Shan Golden Bridge Biological Technology Co. Ltd, China)

**Revision:**

..... DAB Substrate Kit (Beijing Zhongshan Golden Bridge Biotechnology Co. Ltd, China)

5. *Statistical analysis*

.....Methods for calculating the sensitivity, specificity and accuracy were based on the methodology provided in Epidemiology (6th edition, edited by Dr. Ray M. Merrill, Jones & Bartlett Learning Company, Burlington, 2012).

**Revision:**

.....Methods for calculating the sensitivity, specificity and accuracy were based on the methodology provided in *Introduction to Epidemiology* (6th edition, by Ray M. Merrill, published by Jones & Bartlett Learning Company, Burlington, 2012).

**6. Table 2 Frequency of autoantibodies against NPM1, 14-3-3zeta and MDM2 in human sera by ELISA**

12(21.4%) **	11(19.6%) **	11(19.6%)*/**	17(30.4%) **
Cut off value, mean±3 SD of NHCs. * $P < 0.05$ and ** $P < 0.01$ (value relative to CLD and NHC).			

**Revision:**

12(21.4%) <sup>b</sup>	11(19.6%) <sup>b</sup>	11(19.6%) <sup>a/b</sup>	17(30.4%) <sup>b</sup>
Cut off value, mean±3 SD of NHCs. <sup>a</sup> $P < 0.05$ and <sup>b</sup> $P < 0.01$ (value relative to CLD and NHC).			

**7. Figure 1 Western blot analysis of representative sera of three anti-TAAs autoantibody assessed by ELISA.** Lane 1, the polyclonal anti-NPM1 autoantibody, anti-14-3-3zeta autoantibody and anti-14-3-3zeta autoantibody were used as a positive control; Lanes 2 and 3, two representative AFP-negative HCC sera which were positive by ELISA also had strong reactivity with 14-3-3zeta recombinant protein in Western blot analysis; Lanes 4 and 5, randomly selected CLD sera and NHC, respectively, with negative reactivity to 14-3-3zeta recombinant protein.

**Revision:**

**Figure 1 Western blot analysis of representative sera of three anti-TAAs autoantibodies assessed by ELISA.** Lane 1, the polyclonal anti-NPM1 autoantibody, anti-14-3-3zeta autoantibody and anti-14-3-3zeta autoantibody were used as a positive control; Lanes 2 and 3, two representative AFP-negative HCC serum samples which were positive in ELISA also had strong reactivity to 14-3-3zeta recombinant protein in Western blot analysis; Lanes 4 and 5, randomly selected chronic liver disease sera and normal healthy control, respectively, with negative

reactivity to 14-3-3zeta recombinant protein.

8. **Figure 2 Analysis to determine the presence or absence of co-expression of antibodies to any combination of two of the three TAAs in AFP-negative HCC, AFP-positive HCC, CLD and NHC.** The height of the bar represents the percentage of sera with co-expression of two antibodies as, e.g., the presence of NPM1 antibody together with 14-3-3zeta antibody, NPM1 antibody with MDM2 antibody, and so on.

**Revision:**

**Figure 2 Analysis to determine the presence or absence of co-expression of antibodies to any combination of two of the three TAAs in AFP-negative HCC, AFP-positive HCC, CLD and NHC.** The height of the bar represents the percentage of sera with co-expression of two antibodies, e.g., NPM1 antibody with 14-3-3zeta antibody, and NPM1 antibody with MDM2 antibody.

9. **Figure 3 Expression of NPM1, 14-3-3zeta and MDM2 in AFP-negative HCC tissues and normal hepatic tissues by immunohistochemistry.** The three polyclonal anti-TAAs antibody was used as a primary antibody to detect their expression in liver cancer and normal hepatic tissues. (A) (B) HCC tissue with a positive staining signal and normal hepatic tissue with negative staining in anti-NPM1 antibody. (C) (D) HCC tissue with a strong positive staining signal and normal hepatic tissue with negative staining in anti-14-3-3zeta antibody. (E)(F) HCC tissue with a strong positive staining signal and normal hepatic tissue with negative staining in anti-MDM2 antibody.

**Revision:**

**Figure 3 Expression of NPM1, 14-3-3zeta and MDM2 in AFP-negative HCC tissues and normal hepatic tissues by immunohistochemistry.** The three polyclonal anti-TAAs antibodies were used as a primary antibody to detect their expression in liver cancer and normal hepatic tissues. (A), (B). HCC tissue with positive staining and normal hepatic tissue with negative staining in anti-NPM1 antibody. (C), (D). HCC tissue with strong positive staining and normal hepatic tissue with negative staining in anti-14-3-3zeta antibody. (E), (F) HCC tissue with strong positive staining and normal hepatic tissue with negative staining in anti-MDM2 antibody.