Reviewer's report

Title: A Novel Panel of Biomarkers in Distinction of Small Well-Differentiated HCC from Dysplastic Nodules and Outcome Values

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Reviewer: Jorge Filmus

Reviewer's report:

In this paper the authors investigate the usefulness of three markers for the differential immunohistochemical diagnosis of hepatocellular carcinoma (HCC) and high grade dysplastic nodules (HGDN). They also investigate whether the expression levels of these markers can be used for prognosis.

Although there are many studies that have investigated the value of GPC3 immunostaining as a diagnostic tool for HCC, the usefulness of Aminoacylase-1 and Sequestome-1 as diagnostic markers is still unknown. The study of these two markers represents the most novel aspect of this manuscript.

In general, the manuscript is poorly written. Language needs extensive revision. In addition, some parts of the paper are difficult to understand and confusing. For example, in the Methods section it says that the tissue microarrays include 597 samples, but in the Abstract and other parts of the paper they talk about 500 samples. It is also not clear whether the initial set of tissue sections and the one used for validation were analyzed as standard size sections or as tissue microarrays.

Unlike previous reports, this study finds that high levels of GPC3 correlate with better prognosis. The reason for this difference is not clear. Could it be possible that the use of tissue microarrays has an impact on their results? It is well known that HCC tumors have areas that are GPC3-positive and areas that are GPC3-negative. The use of the small sections required by microarrays might increase the number of false negatives. The authors should perform additional prognosis studies by using standard-size tissue sections with at least a proportion of the tumor samples.

Minor criticisms:
- What do the asterisks/dots in Fig. 1B represent?
- In the discussion the authors state that “alterations of GPC3 were also reported in SGBS and lung squamous cell carcinoma”. It should be clarified that in lung tumors there is a change in expression levels, whereas in SGBS GPC3 displays loss-of-function mutations.

Level of interest: An article whose findings are important to those with closely related research interests

Quality of written English: Not suitable for publication unless extensively edited
**Statistical review:** Yes, but I do not feel adequately qualified to assess the statistics.

**Declaration of competing interests:**

I work as a consultant for a Company that sells anti-GPC3 antibodies