Reviewer's report

Title: Microvessel density by automated analysis from regenerative nodule to small hepatocellular carcinoma - approach with CD105 and CD34 immunoexpression.

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Reviewer: Eleni Mayson

Reviewer's report:

Review of manuscript titled: Microvessel density by automated analysis from regenerative nodule to small hepatocellular carcinoma - approach with CD105 and CD34 immunoexpression.

Thank you for the opportunity to review this article.

I found it to be an interesting article which looks at the quantitation of CD34 and CD105 immunohistochemical markers as reflections of microvascular density in three types of lesions: regenerative nodules (as seen in cirrhosis), dysplastic nodules (as a precancerous lesion) and hepatocellular carcinoma. The results showed that median CD34 expression highest in hepatocellular carcinoma, and therefore more indicative of the malignant nature of a lesion. Median CD105 expression was higher in regenerative nodules than in the other lesions, and the authors postulated that this may be due to the hypoxia in these lesions resulting in neovascularisation. There was no difference in expression of these antibodies in the dysplastic nodules.

DISCRETIONARY REVISIONS
None

MINOR ESSENTIAL REVISIONS

• English grammar needs to be reviewed throughout the article, with particular attention to accurate use of the singular and plural, as well as conjunctions eg: “the” and “a”. This will help improve the flow of the manuscript and make it more easily understood.

MAJOR COMPULSORY REVISIONS

1) MVD measurement:
   o I think it would be helpful to explain in detail how the MVD is calculated by the software, given that this is the major endpoint of measurement. I have a haematological background, and so I am unfamiliar with what method this software uses to calculate the MVD.

   o What are some of the limitations of using this software? How sensitive is it? Is it expensive? What QC (quality control) exists and how readily available is this software? Is it standardised across institutions?
2) Tissue used
   o What measures were taken to ensure viability of tissue prior to processing?

3) Antibodies used
   o What internal and external QC is there with the author’s methods?
   o What is the sensitivity and specificity of each antibody?
   o What do the positive and negative controls consist of?
   o What are some of the limitations of the method used?

4) Discussion
   o The authors state that CD105 is more specific than CD34 because it reflects neovascularisation associated with hypoxic tissue. Does the viability of explanted tissue (and the possibility of hypoxic damage) affect these results?
   o CD34 and CD105 has demonstrated prognostic significance in non-hepatic tumours, but what firm evidence is there that it reflects prognosis in hepatic tumours?
   o What is the evidence that MVD quantification confers prognostic significance in hepatic tumours, given that they are highly vascular tumours in vivo?
   o Do in vitro results of these markers, correlate with in vivo prognosis? If so, what aspect of prognosis?

I think the authors need to reword the conclusion and discussion to clarify their findings based on the data presented in their study. They can then substantiate their findings with evidence from other studies.

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

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

**Statistical review:** Yes, but I do not feel adequately qualified to assess the statistics.

**Declaration of competing interests:**

I declare that I have no competing interests