Peer Review File
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Reviewer A

Thank you for your submission. Your manuscript presents valuable insights into the risk factors for tumor thrombus in patients with hepatocellular carcinoma (HCC). However, there are several points that require further clarification:

Comment 1：Measurement of Ki-67: Could you provide more details on the method used for measuring Ki-67 levels? This information is crucial for understanding the validity of your findings. To predict the presence of MV, Ki67 should be measured before treatment. It is not recommended to perform tumor biopsy in case of suspecting HCC because of the risk of dissemination.

Reply 1: No problem, we added some data. The Ki-67 index is determined by the proportion of Ki-67 positive cells in the total number of cells, when the nuclei and/or cytoplasm of Ki-67 positive cells are tan or brownish yellow. Ki-67 specific classification is: < 10% as negative (-), 10% to 19% as weak positive (+), 20% to 49% as positive (++), and more than 50% as strong positive (+++).

Yes, we strongly agree with the opinion of the reviewers. It has been reported in the literature that puncture biopsy in liver can cause a 1% chance of implantation, but successful resection or ablation of the implant site does not affect patient survival.（Silva MA，Hegab B，Hyde C，et al. Needle track seeding following biopsy of liver lesions in the diagnosis of hepatocellular cancer：a systematic review and meta-analysis. Gut, 2008, 57(11)：1592-1596.）

Comment 2：Clinical Decision Making: How feasible is it to use Ki-67 levels in clinical decision-making for HCC patients? Additionally, does the presence or suspicion of Microvascular Invasion (MVI) alter the treatment approach in your study? For instance, would the treatment strategy differ for resectable cases or those with distant metastases, regardless of MVI status?

Reply 2: The Ki-67 expression level after surgery can provide an important reference for the postoperative treatment plan. If the Ki-67 expression level is high, it highly indicates the presence of MVI, even if MVI is not indicated in the pathological report (the current seven-point sampling method for pathology has certain limitations).

Our study is retrospective, the presence or absence of MVI will affect the treatment plan of patients. If MVI exists, the probability of PVTT will be higher, and the formation of PVTT often indicates that patients have lost the opportunity for surgery. Therefore, we should advise the invasion ability of tumors at an early stage to provide important reference for clinical treatment plan.

Comment 3：Additional Explanation Required: Your study would benefit from a more detailed discussion of these points, particularly the practicality of using Ki-67 as a predictive marker in

clinical settings and the implications of MVI on treatment choices.

Reply 3: We added some detailed discussion, particularly the practicality of using Ki-67 as a predictive marker in clinical settings and the implications of MVI on treatment choices.

Ki-67 is a sensitive and highly specific cell marker at proliferative stage, which is an important indicator reflecting the proliferation degree and biological behavior of tumor cells. The higher the positive rate of the marker, the faster the tumor growth, the worse the tissue differentiation, and the worse the prognosis. The expression level of Ki-67 can provide accurate, effective and objective technical means for evaluating the malignant degree of tumor and the efficacy of anti-tumor drugs. (see Page 7, line 210-216).

MVI is an independent risk factor affecting the recurrence free survival time and the overall survival time of liver cancer, and is also an important clinicopathological index significantly related to the invasion and metastasis of liver cancer. If MVI can be predicted before operation, higher requirements should be put forward for the operation, emphasizing the tumor-free operation and radical resection. For postoperative liver cancer with MVI, the commonly used and effective method is to transhepatic arterial chemoembolization to further kill residual microcarcinomas and improve postoperative survival rate. (see Page 6, line 192-199).

Looking forward to your response and additional details to enhance the clarity and applicability of your findings.

Reviewer B

Line 49: what are the tumor thrombus grades?

Reply 1: the grade of tumor thrombus refers to the classification according to whether there is MVI and the presence of PVTT. The larger the thrombus range, the higher the grade.

Line 91: Are you more interested in MVI or tumor thrombus

Reply 2: we're more interested in MVI, which is a type of tumor thrombus.

Line 103: What is this criteria? and can you include a reference for this?

Reply 3: diagnosis includes image-based diagnosis, pathologic diagnosis, and laboratory biomarkers. In most at-risk patients，including those with HBV infection or cirrhosis from any etiology, the diagnosis of HCC should be based on noninvasive imaging criteria or pathology. Biomarkers, such as AFP, are not sufficiently accurate to make a diagnosis of HCC.（Singal AG, Llovet JM, Yarchoan M, et al. AASLD Practice Guidance on prevention, diagnosis, and treatment of hepatocellular carcinoma published correction appears in Hepatology. Hepatology, 2023;78(6):1922-1965.）

Line 110: Did all HCC patients have cirrhosis? Any information on the etiologies of liver disease?

Reply 4: portion of patients had cirrhosis, 101 patients had cirrhosis (Eighty-nine of the patients had cirrhosis caused by Hepatitis B virus infection).

Line 126: How did you differentiate bland vs tumor thrombus? AVENA criteria could be useful to diagnose and differentiate bland versus tumor thrombus

Reply 5: bland thrombus is usually enhanced on enhanced CT or MRI images, while cancer

embolus is enhanced.

Line 156: Did all patients with PVTT also have MVI?

Reply 6: yes, all patients with PVTT also have MVI.

Line 175: Would the etiology of liver disease (hepatitis B, C, MASL, alcohol, etc influence this findings

Reply 7: different etiologies may affect the results, so we will conduct a comparative analysis of liver cancer caused by non-viral hepatitis in future studies.

Line 202: Isn't predicting PVTT a little late? If we predicting vascular invasion, I believe MVI should be the target, not PVTT. Also, if using Ki-67 index, it suggest most patients with HCC would have had treatment for HCC for you to obtain the ki-67 level; which defeats the purpose of predicting PVTT

Reply 8: yes, i agree with the opinion of the reviewer. Our target is MVI, and PVTT is only used for reference comparison. The purpose of the relationship between Ki-67 index and PVTT is to see whether the relationship between Ki-67 index and PVTT will vary with the progression of tumor thrombosis.

Reviewer C

The authors concluded that tumor diameter, Ki-67 expression level, and peritumoral enhancement are good indicators to predict tumor thrombus in patients with HCC. This paper provides some beneficial clinical information; however, some problems must be clarified

Comment 1： Ki-67 expression level can be determined only from histopathological examination. Can it be defined from a liver biopsy specimen? How many patients did you define from liver biopsy specimens? Were the results of Ki-67 expression identical in liver biopsy and resected specimens? If Ki-67 expression level can be judged only after liver resection, postoperative predictive factors to predict microvascular invasion (MVI) are desirable.

Reply 1: yes, Ki-67 expression level can be defined from a liver biopsy specimen, and the expression of Ki-67 in liver biopsy and excision specimens was the same, 34 cases in this study were confirmed by needle biopsy. （Chopra S, Dhall D. Pathologic Diagnosis of Well-differentiated Hepatocellular Lesions: A Practical Approach to Diagnosis With Particular Focus in Core Needle Biopsies and Utilization of Ancillary Techniques. Adv Anat Pathol. Published online June 2, 2023. doi:10.1097/PAP.0000000000000402）.

Comment 2：Does Ki-67 expression add value to traditional histological findings (i.e., tumor differentiation, capsule formation, or capsular invasion).

Reply 2: Ki-67 expression has a certain reference value for traditional histological findings (such as tumor differentiation, envelope formation or envelope invasion), and the higher the expression level is, the more invasive behaviors such as envelope invasion are likely to occur.

Comment 3：Des-γ-carboxy prothrombin (DCP) is one of the well-known predictors of MVI. How about preoperative DCP levels?

Reply 3: yes，DCP is one of the well-known predictors of MVI, the limitation of this paper is that DCP is not taken as an analytical factor in this study, and our data suggest that DCP levels are significantly higher than in patients without HCC.