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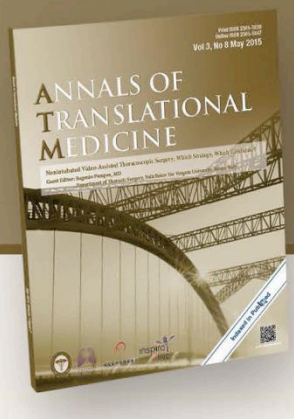

Peer Review File

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Reviewer A:

This study is a retrospective study of a large number of patients and is interesting and clinically meaningful. However, there are several weak points that need to be corrected.

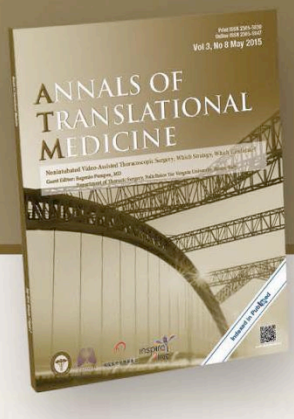
Major concerns:

1. First, the definition of splenomegaly in the method of this study is not clear. The criteria presented by the authors are considered to be one of several criteria for splenomegaly with hypersplenism. Therefore, authors should describe the definition of clear splenomegaly. If the authors classified splenomegaly with hypersplenism, the plt. number in Table S1 is incorrect.

Response: Thanks for your nice suggestions. As we have described in the Methods section: Patients who suffered from splenomegaly with hypersplenism that was classified as greater than class I (spleen enlarged beyond left subcostal margin and palpable) or hypersplenism with a concurrent WBC count of less than 3.0×10^9/L and PLT count less than 80×10^9/L; or grade I or greater splenomegaly with a WBC count of less than 2.0×10^9/L or a PLT count below 50×10^9/L were treated by splenectomy. The splenomegaly was defined as spleen enlarged beyond left subcostal margin and palpable. Some patients were accompanied with splenomegaly without hypersplenism, and the others were with splenomegaly and hypersplenism. Therefore, the PLT number can be greater than 50.

2. The authors recently published a scoring system that predicts the prognosis of total bilirubin, AFP, tumor diameter, and satellite lesions as independent factors affecting OS in a study that analyzed nearly the same patient population over a similar period. However, some of the independent factors affecting the OS in this study are inconsistent. What do you think is the reason?

Response: Thanks for your nice work. In this study AFP and tumor diameter was independent factors affecting OS, yet total bilirubin was not. In another study we previously published, total bilirubin, AFP, tumor diameter, and satellite lesions were all independent factors affecting OS. This current study was conducted in in 6 hospitals including Eastern Hepatobiliary Surgery Hospital, Affiliated Hospital of Binzhou Medical College, Wenzhou People's Hospital, Affiliated to Fujian Medical University, the Second Affiliated Hospital of Wenzhou Medical University, and Qingdao Sixth People's Hospital from 2002 to 2012. However, in the other study we previously published, a scoring system was constructed in Eastern Hepatobiliary



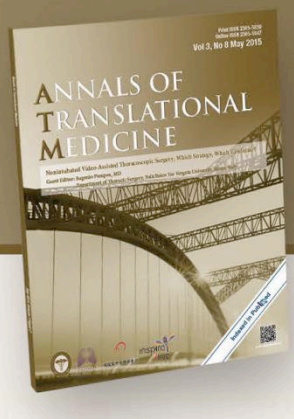
Surgery Hospital and validated in other 3 hospitals including Sun Yat-sen University Cancer Centre, the Affiliated Tumor Hospital of Guangxi Medical University, and the West China Hospital. The scoring system was consolidated in 4 major centers in China, and the findings in this study also validated that AFP and tumor diameter were still prognostic factors for survival. The different databases may be the reason of the difference in the prognostic factor analysis. In addition, all the two studies are retrospective studies with potential bias, which can also explain for this pretty tiny difference in statistical analysis.

3. Is there any reason to analyze only the thickness of splenic hilum among the imaging indicators that diagnose splenomegaly? According to recent reports, composite indicators reflect the actual splenic volume better than single indicators, and spleen max. There are reports that height reflects best. Therefore, it would be better to analyze using more accurate indicators

Response: Thanks for your nice suggestions. We agree with you that some composite indicators may better reflect the actual splenic volume, for example the maximal length, vertical height and hilum thickness. However, the data of maximal length and vertical height was not recorded in our database, and, if necessary, we can analyze these indicators in future studies. In addition, multiple studies have also reported that thickness of splenic hilum can be an indicator for splenomegaly (PMID: 29460042, 28800952).

4. According to another author's paper, among patients with HCC with PVTT, patients with thrombocytopenia had a longer OS than those without thrombocytopenia. In HCC patients, thromboytopenia is caused by hypersplenism accompanied by splenomegaly. If so, this is a completely contrary conclusion to the authors' findings. The authors should explain this difference.

Response: Thanks for your nice work. In the paper of 'YQ Cheng' our team published previously, platelet counts were divided into three categories, the levels ranging from 100 to 300×10^9/L, <100×10^9/L and >300×10^9/L, and they were defined as normal range, thrombocytopenia and thrombocytosis, respectively. It is just the PLT count that was analyzed with the prognosis, and the results found that thrombocytopenia (<100×10^9/L) was a protective factor of OS. However, in this current study, splenomegaly in patients with HCC and PVTT was a complicated pathological process, which may be caused by the portal hypertension of these patients. It is not just the decrease of PLT count. This rather complicated process of splenomegaly in patients with HCC and PVTT may explain this difference. In addition, in patients with splenomegaly, the PLT number can be very low, and lower PLT can be an indicator for poor liver function, thus resulting in suboptimal prognosis for the PVTT patients enrolled in this study.



5. The median survival time of splenectomy patients with splenomegaly among HCC with PVTT patients is twice as long as those without splenectomy (14.0 mons vs. 6.3 mons). These results suggest that splenectomy must be performed in patients with splenomegaly. What do the authors think? Also, the median survival time of patients undergoing splenectomy is even longer than those without splenomegaly. (14.0 mons vs. 12.4 mons). How can you explain these results?

Response: Thanks for your nice suggestions. This study showed that splenectomy can improve the prognosis for PVTT patients with splenomegaly, and splenectomy may act as an excellent treatment option for these patients. Nevertheless, whether splenectomy must be performed in patients with splenomegaly needs to be validated in datasets with more samples or prospective studies.

As for that the median survival time of patients undergoing splenectomy is even longer than those without splenomegaly. There are some reasons explaining this phenomenon: first, the occurrence of splenomegaly will prompt the doctors to perform the surgery earlier, which may lead to the better prognosis; second, according to the clinical practice, post-operation follow-up and surveillance can be more careful and regular for patients undergoing splenectomy, which may also result in the survival benefits; third, the potential bias of retrospective studies may be responsible for these statistical findings.

Minor concerns:

1. The table of basic characteristics is very important information, so it is better to include it in the results rather than supplemental materials.

Response: Thanks for your nice suggestions. We have included table of basic characteristics (Table S1-2) in the results section (Table 1-2).

2. Please, describe clearly whether the values in each table are mean or median.

Response: All the continuous data in each table were reported as medians with interquartile range (IQR), and we have added the denote in the corresponding tables.

3. Please correct the typographic error in the manuscript. (Ex. Page 6. suffered from splenomegaly with splenomegaly).

Response: Thanks for your nice suggestions. We have corrected the mistake (**Page 6, Line 29**).

4. Several references are incorrectly formatted and need to be corrected. (Ex, ref 2,6,8 ……)

Response: Thanks for your nice suggestions. We have corrected the references.

Ref) Hepatology. 2019; 69:2076-2090

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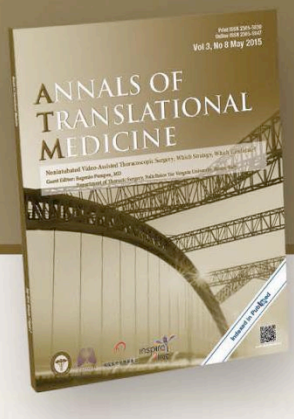


J of Gastro and Hepatol 2019; 34:1214–1221

Reviewer B:

Thank you for allowing me to review the manuscript. Chai Z, et al. investigated the impact of splenomegaly with or without splenectomy on long-term survival of HCC patients with portal vein tumor thrombus (PVTT) after liver resection (LR).

It's very interesting for surgeons who believe splenectomy is an important procedure to improve the prognosis of HCC patients with cirrhosis. However, it has some problems to be clarified. Here below my concern.

Specific comments:

1. The author checked only the prognosis and risk factor by PSM, which sometimes makes the readers boring. How about showing the direct mechanism that the patient who underwent splenectomy has a better prognosis, or discuss the relation between splenectomy/splenomegaly and prognosis, or liver function.

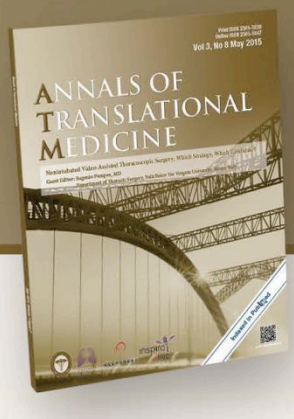
Response: Thanks for your nice suggestions. In the discussion section, there are two findings in our study. The first one was 'splenomegaly was negatively related to the OS and RFS rates of HCC patients with PVTT treated with liver resection'. Next, we have explained the underlying mechanism why splenomegaly was negatively associated with prognosis: 'The reason might be portal hypertensive splenomegaly is usually caused by portal hypertension which can induce hypersplenism. hypersplenism would lead to decreased WBC and platelet counts, which can cause coagulopathy and lower the body's resistance to infection' (**Page 11, Line 4-11**).

The second one was 'splenectomy can improve the survival outcomes for patients with PVTT and splenomegaly'. Next, we have explained the underlying mechanism: 'splenectomy can restore lymphocyte function and induce tumor regression. splenectomy may play a prophylactic role against HCC recurrence after liver resection' (**Page 11, Line 22-27**).

2. Does the splenectomy improve the liver function or immunity against cancer recurrence? Was there any difference between these factors between before and after splenectomy in the HCC patients with splenomegaly? Why do the patients with splenectomy have a low recurrence after hepatectomy?

Response: Thanks for your nice suggestions. Splenectomy may help to improve liver function, nutritional metabolism, and Child–Pugh scores and splenectomy expanded the indications of liver resection and increased RFS.

The reason for that patients with splenectomy have a low recurrence after hepatectomy might be splenectomy can increase the number of natural killer (NK) cells, reduce transforming growth factor (TGF)-β1 expression and alter the immune response to against cancer due to the modulation of CD4+ and CD8+ T cells



3. About splenectomy, when did the patients receive splenectomy? The author wrote, "... had undergone splenectomy before hepatectomy" on page 7, but "by synchronous splenectomy" on page 8. This is a critical mistake.

Response: Thanks for your nice work. This is a mistake, and it should be splenectomy before hepatectomy.

4. The author should define "thickness of splenic hilum" clearly, which seems the independent risk factor. And the meanings of it should be discussed.

Response: Thanks for your nice suggestions. "thickness of splenic hilum" was defined as dimension measured in central part of the hilum perpendicularly to the long axis of the spleen based on the CT or MRI testing, and we have added the description (**Page 7, Line 9-12**).

In addition, the meanings of thickness of splenic hilum has been discussed as follows: notably, the thickness of splenic hilum has been reported to an indicator for splenomegaly, and was also an independent risk factor for survival outcomes in this study. The thickness of splenic hilum acts a novel prognostic factor for survival in patients with HCC and PVTT, which needs to be taken into more consideration in the clinical practice for this group of patients (**Page 10, Line 26-29**).

5. The point that the author checked only the patients with PVTT should be highlighted clearly.

Response: Thanks for your nice suggestions.

As we have described in the Introduction, 'for HCC patients with PVTT, the tumour thrombus may block the blood flow of the portal vein system, and induce portal hypertensive splenomegaly, which may influence the survival outcomes of this group of patients'. It is of great significance to explore the specific influence of splenomegaly on these patients and the impact of splenectomy.

In addition, as we have included in the Discussion section, HCC with PVTT are always combined with decompensated liver function caused by liver cirrhosis. And patients with decompensated liver function pone to accompany with portal hypertension which lead to hypersplenism and influences the outcome of curative treatment. Therefore, it is interesting to explore the effect of splenomegaly with or without splenectomy on the survival outcomes of these patients.