Reviewer 1:

1. Please note that ElastPQ, which is a proprietary software of Philips, is basically an ARFI technique and not a "new shear wave-based elastography" as stated in the introduction and the discussion. On this aspect, please check and cite:

Barr RG et al. Elastography Assessment of Liver Fibrosis: Society of Radiologists in Ultrasound Consensus Conference Statement. Radiology 2015; 276(3): 845-61;

Ferraioli G et al. WFUMB guidelines and recommendations for clinical use of ultrasound elastography: Part 3: liver. Ultrasound Med Biol 2015;41(5):1161-79.

Response: Thank you for your suggestions. The corresponding statements in the introduction and discussion have been revised, and the references you mentioned have been cited.

2. Please, note that the use of ElastPQ in the clinical practice is very well-established! The seminal papers should be cited:

Ferraioli G et al. Point shear wave elastography method for assessing liver stiffness.World J Gastroenterol 2014;20(16):4787-96;

Ma JJ. Assessment of liver fibrosis with elastography point quantification technique in chronic hepatitis B virus patients: a comparison with liver pathological results. J Gastroenterol Hepatol 2014; 29(4):814-9.

Response: We agree with you that the use of ElastPQ in clinical practice is well established, and the references you mentioned have been cited.

3. The references given to support the statement that macrophages play a pivotal role in liver fibrosis are not recent. Please, reword or give more recent references.

Response: "More recently" has been revised to "more interestingly", and the references have been updated.

4. Give reference to the statement "few data pertaining to the evidence of changes in fibrotic liver stiffness after splenectomy at different pathological stages from ElastPQ is available" otherwise state that no data are available.

Response: "Few data" has been revised to "no data".

5. Materials and Methods Were both probes used or only one? This is very important to know since different frequencies give different values for the same degree of stiffness.

Response: The original statement indicated that the system itself was equipped with two transducers, C5-1 (1–5 MHz) and L9-3 (3–9 MHz). In the current study, only the C5-1 was used. The original description has been revised to "equipped with an ElastPQ feature and two transducers, C5-1 (1–5 MHz) [used in this study] and L9-3 (3–9 MHz) [not used in this study]."

6. Discussion Give references for the statement "However, to our knowledge, until now few literature resources with respect to the relationship between the liver stiffness measurement via ElastPQ and liver fibrosis stages are available" (see comment above: the use of ElastPQ in the clinical practice is very well-established! The seminal papers should be cited:

Ferraioli G et al. Point shear wave elastography method for assessing liver stiffness.World J Gastroenterol 2014; 20(16):4787-96;

Ma JJ. Assessment of liver fibrosis with elastography point quantification technique in chronic hepatitis B virus patients: a comparison with liver pathological results. J Gastroenterol Hepatol 2014; 29(4):814-9. Since then, several articles have been published).

Response: We agree with you that the use of ElastPQ in clinical practice is very well established. Our original statement "however,…are available" is incorrect. In consideration of your comment with Comment 2 from Reviewer 2, we deleted the first and second paragraphs in the discussion, making the discussion start with the 2nd sentence of the original Paragraph 3. Furthermore, the mentioned references have been cited.

7. It is not correct to state that "In this study, a trend that splenectomy can delay the progression of early liver fibrosis (especially F1) was detected". In fact, elastography assesses the stiffness which is directly related to liver fibrosis but may change also for other factors, including the quantity of blood in the portal vein. After splenectomy there is a reduction of portal blood flow. On this aspect, in the results it stated that "For the nine rabbits with F1 liver fibrosis (five in splenectomy group vs. four in sham group), the increase of ElastPQ values was delayed in the splenectomy group

compared with that in the sham group during a period of 10 weeks following operations" but no information is given about the histology. Was it improved as well?

Response: Dear reviewer, thank you for pointing out the shortcoming of this manuscript. We know that some factors may influence the liver stiffness measurement (LSM), such as the changes in the portal vein pressure following splenectomy. However, please keep in mind that the purpose of this study is to investigate whether the LSM will be delayed after splenectomy. Splenectomy is an intervention that was used for grouping. If the portal vein pressure or quantity is comparable between groups or before and after splenectomy, another intervention should be introduced, such as vein fluid infusion. In this case, two or more interventions will further confound the results. To minimize bias, two examiners were invited, the blinding method and randomized selection were used, and the success of the LSM was strictly defined; all of these approaches may balance the bias from the changes after splenectomy to some extent.

No information is given about the histology following splenectomy for F1 liver fibrosis rabbits. We provide an explanation as follows:

Based on the histological assessment before and after splenectomy, there is no obvious histological improvement; however, the LSM was significantly different. The paradoxical results can be attributed to the categorical nature of fibrosis staging, while the LSM is a continuous variable. While the LSM was improved, the extent was not sufficient to alter the fibrosis staging, and the histological assessment was unchanged. As a result, we used the word "trend" to politely note that splenectomy can delay the progression of early liver fibrosis.

8. It is preferable not to state that "So far, a number of clinical researches have clarified the feasibility, safety, and effectiveness of splenectomy for liver cirrhosis patients with hypersplenism, suggesting that patients will benefit in terms of short- and long-term outcomes". This issue is still controversial and it should be presented like so [Boyer TD, Habib S. Big spleens and hypersplenism: fix it or forget it? Liver Int. 2015 May;35(5):1492-8]. I would like to underline that it has been shown that the spleen-derived macrophages have a positive role in lung inflammation, thus to recommend splenectomy without any doubt is not advisable [Venosa A, et al.

Protective role of spleen-derived macrophages in lung inflammation, injury, and fibrosis induced by nitrogen mustard. Am J Physiol Lung Cell Mol Physiol. 2015 Dec

Response: This is a very interesting topic. Although some papers on laparoscopic splenectomy for patients with hypersplenism were published from our institution, splenectomy for these patients remains controversial. Some evidence has supported the role of splenectomy for these patients; however, the evidence has not been well balanced. Additionally, the statements in the article you mentioned also indicated that hypersplenism in most patients should be considered a laboratory abnormality that is not treated or further considered. Therefore, we deleted the statement "So far, a number of clinical researches have clarified the feasibility, safety, and effectiveness of splenectomy for liver cirrhosis patients with hypersplenism, suggesting that patients will benefit in terms of short- and long-term outcomes." The original statement was revised as:

"Although splenectomy was performed for patients with hypersplenism in some institutions[1, 2], hypersplenism in most patients should be considered as a laboratory abnormality that does not require treatment or further consideration[3]. However, a previous well-designed study indicated that splenectomy attenuated murine liver fibrosis considering that hypersplenism stimulates hepatic accumulation of macrophages[4]. Therefore, splenectomy remains controversial for patients with hypersplenism. In the present study, splenectomy was only used for grouping rabbits and then exploring whether splenectomy at different liver fibrosis stages will delay or reverse the progression of liver fibrosis."

Dear reviewer, you mentioned that spleen-derived macrophages play a positive role in lung inflammation and that to recommend splenectomy is not advisable. I agree with your point of view. However, I want to emphasize that spleen-derived macrophages have different roles in different pathological conditions. A previous well-designed study indicated that splenectomy attenuates murine liver fibrosis and that hypersplenism stimulates hepatic accumulation of macrophages5. Kim E compared mice that did and did not undergo splenectomy in acute ischemic brain injury, and reported the involvement of spleen-derived total macrophages in acute infarct development6. Izci Y. indicated that removal of the spleen may decrease the production of mononuclear cells and thus hinder or relieve the inflammatory reaction after cerebral ischemia/reperfusion injury. Splenectomy may be a prophylactic treatment method for cerebral ischemia7. Venosa A emphasized the

protective role of spleen-derived macrophages in lung inflammation, injury, and fibrosis induced by nitrogen mustard8. As a result, the role of spleen-derived macrophages is heterogeneous. Their role in liver fibrosis requires an in-depth mechanism study, which is not the main purpose of the current study. Splenectomy is only used to compare groups of rabbits in this study. The main purpose of the study was to evaluate the performance of ElastPQ and its longitudinal application in liver fibrosis conditions.

Our hypothesis of our role of spleen-derived macrophages is considered based on the previous studies6-9. We used subjective mood when discussing spleen-derived macrophages (the 6th paragraph of discussion). We presented the statements here to show some clues about the liver fibrosis mechanism for ourselves and other researchers.

The topic of spleen-derived macrophages in liver fibrosis is very interesting and merits in-depth basic studies to confirm its future role.

Reviewer 2:

This manuscript (ms) shows data from a new method of LSM; ElastPQ. This study is well powered and the statistics are appropriate. A strength of this ms is that the model of fibrosis is improved from a previous publication. This ms shows no benefit of splenectomy in this model. A strength of this ms is that the measurements are very careful to ensure the maximal precision by using two experts for biopsy score and for LSM. The data is interpreted very well . I like the discussion of fibrosis resolution and macrophages. A strength of this ms is that it is well written and the data are carefully interpreted.

Revision comments:

1. The exception regarding writing quality is that parts of the Discussion need improved English, in particular the 4th paragraph and 8th paragraph must be re-written. Also, the phrase , “can be alleviated “ is unclear and must be explained [Discussion para 7]. An example in para 8 is that the phrase beginning, “recently confirmed ….. “ is unclear and must be explained. The last 3-4 sentences of para 8 need to be clarified [Discussion para 8].

Response: The entire discussion section was edited for language and some unclear statements have been clarified.

The statement"recently confirmed…"has been changed to: "Based on a study of monocytes derived from bone marrow (BM)[5] as well as studies by Swirski FK and other researchers, there are numerous monocytes in the spleen that could be mobilized in pathological states. As a result, the spleen can be considered a monocyte reservoir[6-8]."

The last 3-4 sentences summarize our presumed hypothesis. We used the subjective mood for these sentences. We presented the statements here to provide some clues about the liver fibrosis mechanism for ourselves and other researchers.

2. The weakest part of this ms is the discussion. The first two paragraphs are introductory and are redundant when placed in the Discussion Section. The second sentence of 3rd para of Discussion is where the Discussion Section should start.

Response: The first two paragraphs have been deleted, and the discussion started with the second sentence of the original 3rd paragraph.

3. Discussion needs to comment on CCl4 fibrosis models and on rabbit models of liver fibrosis.

Response: A CCl4-induced liver fibrosis model on rabbits is well-established12-15. Additionally, the modified modeling method in this study was detailed in the section of materials and methods section. This modified method can decrease mortality. It is easy and convenient to induce liver fibrosis in rabbits using the modified method. Therefore, there is no need to provide extra comments on this model.

4. Please explain in the ms why collagen IV was chosen as a measure. General comments: It would have been interesting to measure blood pressure, in particular portal pressure, in these animals. This ms would be greatly strengthened by adding a direct comparison with TE.

Response: Because collagen IV is a Class I biomarker of liver fibrogenesis16, we chose it as a control when performing ROC analysis.

We agree with you very much that measuring the portal vein pressure and comparing ElastPQ and TE would strengthen our results. However, considering the long-time required for liver fibrosis modeling and the topic of longitudinal evaluation of the LSM by ElastPQ in this study, it is very difficult to redo the experiment.

Reviewer 3:

The manuscript `Non-invasive evaluation of liver stiffness after splenectomy on CCl4-induced liver fibrosis in rabbits` by Wang MJ et al. is an interesting and well-elaborated paper presenting study of utilizing CCl4-induced liver fibrosis with liver stiffness measurement. The approach is not new and model might be most likely considered as reliable for the liver research. Sonoelastography should strongly expand a potential of the longitudinal experiment. However, some minor corrections might improve the paper quality via focused discussion/correction the following points:

1. Ultrasound image would appreciated, best option would to present side-by-side comparison sonoelastography–histology for fibrosis grades.

Response: Thanks you for this suggestion. The US images are presented in the manuscript.

2. Why splenectomy was included to the experiment? Authors claim that `Splenectomy is one of surgical interventions for liver cirrhotic patients with hypersplenism`, but how hypersplenism was evaluated in included animals? Did animals demonstrated signs, lab tests relevant to hypersplenism? Was the portal hypertension and visceral blood flow redistribution considered? This is major point in the liver stiffness increasing mechanism. Statement that `splenectomy can delay the progression of early liver fibrosis` is very confusing.

Response: This is a very interesting topic. Although some papers on laparoscopic splenectomy for patients with hypersplenism were published at our institution, hypersplenism in most patients should be considered as a laboratory abnormality that is not further treated or considered. However, a previous well-designed study indicated that splenectomy attenuates murine liver fibrosis with hypersplenism, stimulating hepatic accumulation of macrophages. Therefore, splenectomy remains controversial for patients with hypersplenism. In the present study, splenectomy was only used for grouping the rabbits and then exploring whether splenectomy at different liver fibrosis stages delays or reverses the liver fibrosis progression. Whether hypersplenism is the proper indication for splenectomy is another topic that is beyond the scope of this study.

Therefore, we deleted the original statement "So far, a number of clinical researches have clarified the feasibility, safety, and effectiveness of splenectomy for liver cirrhosis patients with hypersplenism, suggesting that patients will benefit in terms of short- and long-term outcomes." The paragraph was revised to state:

"Although splenectomy was performed for patients with hypersplenism in some institutions1-3, hypersplenism in most patients should be considered a laboratory abnormality that is not treated or further considered4. However, a previous well-designed study indicated that splenectomy attenuated murine liver fibrosis with hypersplenism, stimulating hepatic accumulation of macrophages5. Therefore, splenectomy remains controversial for patients with hypersplenism. In the present study, splenectomy was only used for grouping rabbits and then exploring whether splenectomy at different liver fibrosis stages would delay or reverse the progression of liver fibrosis.

Once more, splenectomy is only used to group the rabbits in this study. The main purpose of the study was to evaluate the performance of ElastPQ and its longitudinal application in liver fibrosis. Liver fibrosis was confirmed by histological examination; however, further tests of hypersplenism were not performed. We know that measurements of the portal vein pressure and lab tests relevant to hypersplenism would strengthen our results. Considering the long time required for liver fibrosis models to develop and the topic of longitudinal evaluation of the LSM by ElastPQ in different fibrosis stages following splenectomy in this study, it is very difficult to redo the experiment.

3. The design of invasive part has many unclear points, bias:

- `To obtain different stages of liver fibrosis at different time intervals, the same surgical process was repeated for the remaining rabbits every two weeks until the 20th week.` - can alter hepatic tissue and evoke fibrosis as well

Response: Dear editor, I am very sorry for the confusion about the experiment process. The statement that the same surgical process was repeated for the remaining rabbits every two weeks until the 20th week was meant to state that during the next 20 weeks, 8 rabbits were randomly selected for surgery every 2 weeks (Table 1). In detail, in the 4th week, 8 rabbits were randomly selected from the 82 rabbits that were chosen for surgery; in the 8th week, 8 rabbits were randomly selected from the

74 rabbits for surgery and so on. This means that in experiment 1, every rabbit only received a single surgery.

- Along with the increase of operation times, it was more and more difficult to get the liver tissue along the original midline incision because of adhesion` - taking tissue samples in one area might bias results be due to the postoperative scars.

Response: Dear reviewer, thank you very much for your concern. Before this experiment, we had performed a preliminary experiment and found that along with the increase in the operation times, the adhesion and scars were moderate (not severe). Even so, we kept in mind that the scars and adhesion may confound the results. Therefore, after preliminary experimentation, we chose chitosan (0.5 mL/surgery) to avoid adhesion. Additionally, in the section on the surgical procedure, we have indicated that along with the increase in the operation times, it was difficult to acquire liver tissue along the original midline incision. In this case, a left or a right subcostal incision was needed.

- Did you measure liver stiffness in biopsy samples for comparison? Did you consider that post mortem stiffness might be strongly altered to be properly compared.

Response: It is regretful that we did not measure the liver stiffness in biopsy samples for comparison

- Authors did the biopsy in the subxiphoid region `to reduce the biases`. And where sonoelastography assessment was performed?

Response: The LSM was measured before biopsy; as a result, if possible, sonoelastography assessment was performed in the subxiphoid region. When the subxiphoid region was unavailable, the region close to the subxiphoid was the alternative region.

- Another relevant bias is giving penicillin intramuscularly in doses of 40 U/rabbit to prevent infection.

Response: Dear editor, this study involved surgery, including splenectomy, biopsy, and sham operation and, in such conditions, is acceptable to use antibiotics to prevent infection.

- Translation issues could be discussed. The reference list might be expanded with newest studies on using sonoelastography in human and using ultrasound in animal models. Some language quality might be improved and spelling corrections to be done.

Response: The entire manuscript has been edited for language, and the references have been updated.

1. Yu H, Guo S, Wang L, Dong Y, Tian G, Mu S, Zhang H, Li D, Zhao S: Laparoscopic Splenectomy and Esophagogastric Devascularization for Liver Cirrhosis and Portal Hypertension Is a Safe, Effective, and Minimally Invasive Operation. J Laparoendosc Adv Surg Tech A 2016, 26: 524-30.
2. Yamamoto N, Okano K, Oshima M, Akamoto S, Fujiwara M, Tani J, Miyoshi H, Yoneyama H, Masaki T, Suzuki Y: Laparoscopic splenectomy for patients with liver cirrhosis: Improvement of liver function in patients with Child-Pugh class B. Surgery 2015, 158: 1538-44.
3. Boyer TD, Habib S: Big spleens and hypersplenism: fix it or forget it? Liver Int 2015, 35: 1492-8.
4. Yada A, Iimuro Y, Uyama N, Uda Y, Okada T, Fujimoto J: Splenectomy attenuates murine liver fibrosis with hypersplenism stimulating hepatic accumulation of Ly-6C(lo) macrophages. J Hepatol 2015, 63: 905-16.
5. Geissmann F, Manz MG, Jung S, Sieweke MH, Merad M, Ley K: Development of monocytes, macrophages, and dendritic cells. Science 2010, 327: 656-61.
6. Swirski FK, Nahrendorf M, Etzrodt M, Wildgruber M, Cortez-Retamozo V, Panizzi P, Figueiredo JL, Kohler RH, Chudnovskiy A, Waterman P, Aikawa E, Mempel TR, Libby P, Weissleder R, Pittet MJ: Identification of splenic reservoir monocytes and their deployment to inflammatory sites. Science 2009, 325: 612-6.
7. Venosa A, Malaviya R, Gow AJ, Hall L, Laskin JD, Laskin DL: Protective role of spleen-derived macrophages in lung inflammation, injury, and fibrosis induced by nitrogen mustard. Am J Physiol Lung Cell Mol Physiol 2015, 309: L1487-98.
8. Kim E, Yang J, Beltran CD, Cho S: Role of spleen-derived monocytes/macrophages in acute ischemic brain injury. J Cereb Blood Flow Metab 2014, 34: 1411-9.