Dear Editor, We would like to thank the editor for giving us a chance to resubmit the paper, and also thank the reviewers for giving us constructive suggestions which would help us to improve the quality of the paper. Here we submit a new version of our manuscript with the title of "Preliminary exploration of the application of Super microvascular imaging in Focal Liver Lesions" which has been modified according to the reviewers' suggestions. We marked all the changes in the revised manuscript. Sincerely yours, Mengna He, MD.

The following is a point-to-point response to the three reviewers' comments.

Reviewer #1

From Greece

1. The technique is very interesting, however the authors proposed 7 subtypes of SMI examination, which seems a little complicated for general use. I think that this technique is in his early phase before adapted to general use. The easier and clinically significant would be to differentiate between benign and malignant disease, however such sub differentiation has not been proposed.

Answer: Thank you for the comments on the paper. The suggestion of dividing the FLLs into benign and malignant group is very valuable and we will do research about this topic further. For the present study, we proposed 7 SMI subtypes because in our research the malignant cases including primary hepatic lymphoma and metastatic lesions, of which the clinical treatment were different from hepatic cellular carcinoma, and hepatic adenoma was also different from other benign lesions. Refer to your suggestions, we add the comparison of the SMI types between the most common malignant FLLs of HCCs and metastatic lesions with the most common benign FLLs of HEs, the results were obviously showed the difference.

2. Another confusing issue is that type II (strip rim type) were found in 8 benign HEs, and in 4 malignant liver metastases from breast cancer.

3. Also one benign adenoma had the same features (type IV, diffuse honeycomb type) as 3 HCCs.

Answer for question 2 and 3: as you mentioned, we also found that some different

FLLs shared the same SMI type, for example, the metastatic lesions from the tumor of breast shared strip rim type (type II) with HE; the other metastatic lesion from pancreatic had the same SMI type of thick rim type (type VI) with the primary hepatic lymphoma; the hepatic adenoma and partial HCCs had the same type of diffuse honeycomb type (Type IV).

We have explained the reason in the discussion part, for metastatic lesions, it is always assumed that their imaging performance is particularly confusing because metastatic lesions can simulate various other kinds of FLLs. For hepatic adenoma (HA), researches about the ultrasound features of HA were relatively rare because of the low morbidity compared to other FLLs, Wong et al had done a retrospective study to analyze the difference of ultrasound and CEUS features between HA and HCC, the results showed that most cases of HAs were manifested as homogenous, rapid and complete enhancement in the arterial phase which is similar to HCC.

Thus, when a FLLs was suspected as a SMI type we classified, we still need to integrate the result with clinical history.

4. This study to be more complete should be accompanied by specificity, sensitivity, negative and positive predictive value (NPV and PPV) and to be compared to other existing techniques. Also should be simplified for clinical use.

Answer: very thanks for your suggestion. As you considered, SMI technique is in his early phase before adopted to general use, for the present study, we want to explore the SMI characteristics of various different types of focal liver lesions, and make a classification, the next research we will evaluated the value of this SMI classification method in the diagnosis of different FLLs especially for benign and malignant ones, and simplify the classification for clinical use.

Reviewer #2

From Germany

Abstract:

1. For the abbreviation of ‘SMI’, authors use ‘Super microvascular imaging’ in the

title, however use ‘Super-micro vascular imaging’ in the abstract. Please keep consistence throughout the text.

Answer: thank you very much for your detailed review, we have unified named it as “Super microvascular imaging” which is for the abbreviation of “SMI”.

2. Authors described various kinds of ‘SMI subgroups’ in their results. However, they did not mention the comparison between CDFI and SMI, so they had no results to support their conclusion as ‘SMI had obvious advantages in vascular visibility for the small FLLs compared with CDFI’.

Answer: we have removed this conclusion from abstract, and put this results in the main body of this article for further discussion.

3. This study only included very small size, especially for FNH, lymphoma and adenoma. So it will be inappropriate for them to concluded as ‘the SMI characteristic of different FLLs were significant different’.

Answer: consider of relative small size of FNH, lymphoma and adenoma, which were also rare in the clinical work, we transferred to compare the SMI types between the most common malignant FLLs of HCCs and metastatic lesions with the most common benign FLLs of HEs..

Introduction:

1. Authors mentioned ‘SMI has the obvious advantage of detecting more slow blood flow and reveal micro-vessels’. Why and How? Any literature to support this theory? Please describe the gold standard as already mentioned.

Answer: a series of studies about the ability to detect more slow blood flow and reveal micro-vessel in the masses of thyroid and breast by SMI have been reported, Priscilla et al evaluated the capability of SMI to visualize the microvascular flow in normal thyroid tissue and thyroid nodules compared with CDFI, and the results showed SMI had better depiction of vessel branching; Jia et al compared the ability to discover penetrating vessels (PVs) of breast cancer between CDFI and SMI, the results showed more PVs were observed by SMI than CDFI. While studies of SMI about FLLs are still rare, the purpose of our study is to investigate the SMI features of FLLs and to analyze the ability to provide additional information for clinical differential diagnosis.

2. Nowadays, CEUS has been gradually recognized as a comparable imaging technique in diagnosis of FLLs, with great accuracy and convenience. Why do we still need SMI, which might not be as sensitive as CEUS in detecting small vascular perfusion? Authors should clarify this in their introduction.

Answer: we have clarified the advantages and disadvantages of CEUS in the introduction part. "Besides, the agents used in CEUS, CECT and CEMRI were foreign bodies, each of them may cause hypersensitivity reactions; secondly, the three techniques take additional economic costs and time consuming, so widely available is difficult."

Methods:

1. Please include gold standard(s) for all examined criteria.

2. How many adenoma, hemangioma and FNH have been biopsied (or surgery performed) and why not the others? 4. '9 lesions were pathologically diagnosed', by operation or by biopsy? Authors should detailed clarify it.

Answer for question 1 and 2:

We have completed the gold standard(s) for CDFI, SMI examinations, and the diagnostic gold standard including pathology and radiology based on the guideline of American College of Gastroenterology (ACG).

9 lesions were pathologically diagnosed by operation including 2 lesions of HE in 2 patients, 5 lesions of HCC in 4 patients, 1 lesion of hepatic adenoma and 1 lesion of primary hepatic lymphoma; 22 lesions were radiological confirmed，including 15 HEs, 5 lesions of metastatic mass and 2 lesions of FNH. Because based on the guideline of American College of Gastroenterology (ACG) [1]，benign FLLs could be diagnosed by imaging, and biopsy may be not recommended for the reasons of hemorrhage especially for some HEs and adenomas.

3. What is authors' detailed definition of 'SMI characteristics of the FLLs'? Any previous research or literature to support this subtypes classification?

Answer: there was only two investigations had been reported: one was reported by Wu et al, SMI clearly demonstrated the typical "spoke-wheel" vascular type of FNH in the liver without using any contrast agent; another one was reported by Lee et al,

the application of SMI in three kinds of 29 FLLs including HE, HCC and FNH and concluded that the SMI types were significant different between FLLs. The latter research classified the SMI type into 8 subtypes, we have referred to the previous classification method and also made some improvements combined with the FLLs of the present study.

5. Why did authors divide the 31 FLLs into small or large FLLs groups by 3.0cm?

Answer: because based on the Guidelines for the Diagnosis and Treatment of Primary Liver Cancer (2011 Edition) proposed by the Chinese Minister of Health[2], a single tumor≤3cm was named small hepatocellular carcinoma (SHCC) for the clinical diagnosis and treatment may be different from HCCs ＞3cm. Thus, we divided the 31 FLLs into small or large FLLs groups by 3.0cm.

6. Authors only compared the vascular visibility between CDFI and SMI? why not between SMI and CEUS?

Answer: because when a patient under a CEUS examination, agents is inevitable, besides, the procedure of CEUS is much more complex than CDFI and not every radiologist can do CEUS technique.

7. What is 'within and between groups comparisons' refer to in 'statistics' part? Please describe more precisely in the groups and statistical methods they used.

Answer: we have corrected it as "The differences of SMI types between the most common malignant FLLs of HCCs and metastatic lesions with the most common benign FLLs of HEs were evaluated by Fisher's exact test".

8. Since the SMI subtype is a relatively subjective observation, the imaging data were analyzed by two experienced radiologists, any inter- or intra- observer biases?

Answer: if any disagreement happened, decision would be made until reached a consensus after consultation.

9. 'The difference of size and year were evaluated by one-way ANOVA test'. Why to compare the 'year'? Any relation to the SMI subtypes?

Answer: the reason for comparing the year just want to analyze if there is any age and sex difference between the groups, no relation to SMI subtype.

10. Authors mentioned 'CDFI mode missed the vascular of 69.2% FLLs in small

group and 11.8% lesions in > 3.0cm group'. The rates seem to be high. For CDFI examination, what were the parameters settings?

Answer: when for CDFI examination, the scale was set as low as possible until at the appropriate level without any Pseudo color flow like color flow spillover, the lowest scale was at 4cm/s, and the flow gain was adjusted high enough until noise emerged.

Results:

1. 'Satisfactory images' were got for all 31 FLLs including CDFI and SMI. However, CDFI mode could not detect blood flow signals in several cases. These seems to be contradictory.

Answer: Despite of 'satisfactory images', CDFI has the limitation to describe slow blood flow and reveal micro-vessel which is important for diagnosis, thus, we want to evaluate whether SMI has the ability to assess micro-vessels.

2. Only small cases were included in each FLLs group, some even with only 1 or 2 cases. How could authors get the results as 'the distribution of SMI types between FLLs were significant different ($P < 0.05$)'?

Answer: we have corrected it as "The differences of SMI types between the most common malignant FLLs of HCCs and metastatic lesions with the most common benign FLLs of HEs were evaluated by Fisher's exact test", and the result showed than $p=0.048, < 0.05$.

Discussion

1. Authors discussed CDFI had many limitations in depicting tumor vessels. Why did they still compare 'the ability of CDFI mode and SMI mode to detect the vascular of 31 FLLs'? Why not compare with CEUS or MRI?

Answer: we want to evaluate whether the novel Doppler technique of SMI has the ability to assess micro-vessels, and whether SMI is better than CDFI. The reason for not comparing SMI with CEUS/MRI is as following: when a patient under a CEUS/MRI examination, agents is inevitable, besides, the procedure of CEUS/MRI is much more complex than CDFI and not every radiologist can do CEUS/MRI technique.

2. 'SMI clearly demonstrated the typical "spoke-wheel" vascular type of FNH'. CDFI

and CEUS also could demonstrate typical “spoke-wheel” vascular type of FNH. SMI seems to have no advantage.

Answer: we have correct is as “this research including two cases of FNH, and both CDFI and SMI manifested as typical “spoke-wheel” type, while without the basic echo of liver parenchyma, the vascular structure by SMI seemed much more clear, which is the same with the previous studies”.

3. What are the ‘typical SMI types’ for FLLs? Especially for HCC or metastasis lesions? Please be more clearly in the description.

4. This study only included 1-2 cases of FNH, lymphoma and adenoma. Also SMI type for lymphoma and adenoma are not typical. How could SMI ‘provide some helpful information’ for the diagnosis of those FLLs?

Answer for question 3 and 4: SMI technique is in his early phase before adopted to general use, for the present study, we want to explore the SMI characteristics of various different types of focal liver lesions, and make a classification. Based on the classification, for HEs, the “typical SMI types” including diffuse dot-like type; strip rim type; nodular rim type, for FNHs in the present study, SMI manifested as typical “spoke-wheel” type, for HCCs, we classified them into diffuse honeycomb type and non-specific type, for metastatic lesions, it is always assumed that their imaging performance is particularly confusing because metastatic lesions can simulate various other kinds of FLLs, just as the present study, the SMI types of metastatic lesions were similar to HE and lymphoma. For the primary hepatic lymphoma and hepatic adenoma, because both of them are relative rare diseases, ultrasound research about these two kinds of disease is rare and the imaging features is still unknown. The present study want to assess whether the novel technique of SMI would provide any useful imaging information about the micro-vessels of them.

Tables 1. All abbreviations in the table require annotations. 2. Please be specific in the table what do ‘SMI type I - VII’ mention to?

Answer: we have added the annotations for all abbreviation and completed the specific type which ‘SMI type I - VII’ mention to.

Figures 1. Figure 4, I did not find anything like ‘honeycombs pattern’ in the figures. 2.

Figure 8, How to differentiate between ‘Type II, strip rim type’ and ‘Type VII, spoke-wheel type’?

Answer: Figure 1 described diffuse dot type. Figure 4 included one case of HCC and one case of HA, we classified them into ‘honeycombs pattern’ based on the rich and thin strip inner micro-vessels assembled like grid. The main difference between ‘Type II, strip rim type’ and ‘Type VII, spoke-wheel type’ is the latter has inner radial vascular Like a wheel.

Reviewer #3

From Italy

1. Authors should report the intra observer and inter-reader agreement to better evaluate the reliability of this new method to detect small lesions.

Answer: if any disagreement happened, decision would be made until reached a consensus after consultation.

参考文献

[1] Marrero J A, Ahn J, Rajender R K. ACG clinical guideline: the diagnosis and management of focal liver lesions[J]. Am J Gastroenterol, 2014,109(9):1328-1347, 1348.

[2] Cong W M, Bu H, Chen J, et al. Practice guidelines for the pathological diagnosis of primary liver cancer: 2015 update[J]. World J Gastroenterol, 2016,22(42):9279-9287.