Point-by-Point Response to peer-review report(s)

Dear Reviewers,

Many thanks for your comments. We are grateful for the time and review undertaken. As requested, please find below a point-by-point response to each issue raised in the reports:

Reviewer #1:
Scientific Quality: Grade B (Very good)
Language Quality: Grade B (Minor language polishing)
Conclusion: Major revision

Specific Comments Addressed:

Introduction:

1. "… with CSPH as the spleen undergoes parenchymal remodelling and fibrogenesis, due to blood pooling in PH (5-7)." SS has been proven to depend on inflammation as well. Given the broad introduction I suggest adding 1-2 lines explaining it. References to cite: 1) PMID: 6206152 – DOI: 10.4254/wjh.v10.i10.731; 2) PMID: 32304009 - DOI: https://doi.org/10.1007/s40477-020-00456-9 .

Response: As per the above comment, the following sentence was added to paragraph 4 of introduction (page 5) and cites the two recommended references: "Interestingly, evidence on patients with chronic hepatitis C infection also suggests that spleen stiffness is dependent on inflammation present in the liver that directly contributes to the pathogenic mechanisms underlying PH[8-9]."

2. "fewer studies have looked at the performance of ElastPQ due to its novelty." Given the fact that the authors are performing measure using the ElastPQ

evaluation protocol, as a general comment, they should compare their findings to those of authors who used the same protocol. Here you find to interesting articles: Evaluation of SS in healthy individuals and study on double blind agreement of measure: PMID: 31054978 – doi:c10.1016/j.aohep.2019.03.004 These authors developed a predictive model using SS measured ElastPQ protocol, and study the use of spleen diameter/area - this may results specifically useful to your introduction/discussion to better explain your results: PMID: 31740162 – doi: 10.1016/j.aohep.2019.09.004

Response: Many thanks for highlighting the above studies that have recently featured within this field. We have edited the discussion to better explain our results given the similarities in study design and objectives. Please see the comment under "Discussion" for further information.

Subject and methods:

1. Authors should clarify the time interval between OGD and SS measurement. Because if the maximum interval is one year, it may be a consistent bias and should be explained as a limitations.

Response: The maximum interval limit of 1 year has been rectified in paragraph 2 of methodology (see below) so that it clearly states this within the methodology. Of note, the median time difference between ultrasound elastography measurements and EGD/HVPG was 4 months.

Paragraph 2 of methodology [study design] (page 7): "The primary analyses were conducted after all patients were recruited. The patients were divided into the following groups: evidence of CSPH (group 1) and no evidence of CSPH (group 2). CSPH was defined either as presence of EV or portal hypertensive gastropathy

(PHG) during an EGD or if patients had invasive procedures where the HVPG pressure ≥10mmHg. Ultrasound elastography measurements must have been undertaken within a maximum of one year of EGD or HVPG measurements."

Response: We agree that 1 year is a somewhat lengthy time interval between spleen stiffness measurements and EGD. This limitation has been highlighted in paragraph 8 of discussion [strengths and limitations] (pages 13-14) in the following format: "This study has some limitations, the most pertinent of which being that we only assessed for presence or absence of PH, rather than degree of PH. Furthermore, an interval gap of one year between spleen stiffness measurements and EGD/HVPG readings may represent a consistent bias within our study due to the considerable length of time between readings. However, it could be argued that the correlation between CSPH and spleen stiffness may be better if there were a shorter time interval proposed."

2. Also, please report the ultrasound machine model (Philips Affiniti 70? IU22?)

Response: Please find the following sentence specifying the machine model in paragraph 3 of methodology [ultrasound and elastography] (page 7): "All patients had to be fasted for up to 6 hours prior to scans. Participants were placed supine with arms abducted away from the ultrasound probes. The Philips Affiniti 70 [ElastPQ] (Philips Medical Systems, Seattle, USA) was used to record liver stiffness measurement and spleen stiffness measurement for each patient." Of note this is also reflected in the abstract methods (page 3): "Liver stiffness, spleen stiffness, spleen diameter and spleen area were measured using the Philips Affiniti 70 [ElastPQ] point shear wave elastography system."

3. Also, authors should better clarify inclusion/exclusion criteria? Did you include patients undergoing non-selective beta blockers? TIPS? Ongoing liver injury?

Response: The protocol for the original study had a broad inclusion criteria. The only patients excluded were those who had a TIPS procedure, were pregnant or had HCC. Given the recent data provided regarding the potential confounding factors of spleen stiffness, we have included the following sentences in paragraph 8 of discussion [strengths and limitations] (page 15): "We did not exclude patients taking pharmacological treatment for PH from the original protocol as it was suspected that non-selective beta blockers and banding of varices would be unlikely to affect splenic measurements[32]. However, the most recent data on cirrhotic patients with high risk varices suggests that taking non-selective beta blockers can affect splenic stiffness[33, 34]. But these studies were undertaken using Fibroscan® and VTQ (Siemens Acuson S2000TM) ultrasound systems and so, further information is still needed in order to confirm that similar findings are present with the ElastPQ."

4. "Ten measurements were taken from the right lobe of the liver and ten measurements from the spleen." Were the measure performed all in the same lobe of the liver? How much distance from the liver capsule? Were the measure performed on the lower/upper/middle portion of the spleen?

Response: We agree that further detail on the elastography measures are needed and these have been added to paragraph 3 of methodology [ultrasound and elastography] (page 7): "Ten measurements were taken from the liver and ten measurements from the spleen. Liver elastography measurements were taken from the right lobe of the liver 2.40 cm (±1 cm) away from the liver capsule. Spleen elastography measurements were taken from the middle aspect of the spleen with homogeneous elasticity with the exclusion of big vessels. The median stiffness and IQR values were recorded. Spleen area and diameter were calculated from 2D images obtained."

Results

1. As a general comment: when the authors report ROC and AUROC, they should not explain it as correlation, but as discrimination. Please correct this concept.

Response: As in agreement with the phraseology, we have amended the following paragraphs to reflect this:

Abstract [Results] (page 3): On univariate and individual performance, platelet count (AUROC 0.846, p-value: <0.001), spleen area (AUROC 0.828, p-value: 0.002) and APRI score (AUROC: 0.827, p-value: <0.001) were the most accurate variables in identifying the presence of portal hypertension.

Paragraph 3 of results [univariate analysis] (page 9): No statistically significant discrimination was found between liver stiffness measured by the ElastPQ and CSPH (AUROC 0.657, p-value: 0.061).

2. Also the authors should report how the logistic regression was performed in the statistical section.

Response: As requested, we have expanded on the logistic regression analysis in paragraph 6 of the methodology [statistical analyse] (page 8)): "A multivariate logistic regression model was built using a stepwise selection to determine the association of spleen area and platelet count and spleen stiffness and platelet count presence of CSPH. It was ensured that the data fulfilled all necessary criteria prior to application of the logistic regression analysis."

Discussion

1. I suggest commenting your results also in the light of the paper cited before (PMID: 31740162 – doi: 10.1016/j.aohep.2019.09.004)

Response: The discussion has been amended in several places to compare our paper to the one highlighted above. Please find below the comments included within the listed paragraph numbers of the discussion section:

Paragraph 2 of discussion (page 11): "Nevertheless, in a recent study which adopted a similar methodology to our own, Giuffrè et al. identified a cut off of <31kPa to rule out the presence of EV of any grade which resonates with our findings[19]."

Paragraph 4 of discussion (pages 11-12): "Splenic area and diameter demonstrated a modest ability to diagnose the presence of CSPH. Previous studies have explored spleen size by consideration of splenic diameter[22], which has shown to have acceptable reproducibility in the context of platelet count/spleen diameter ratio. However, to our knowledge, there has only been one other study which has considered spleen area as a potential non-invasive diagnostic parameter. In this study, Giuffrè *et al.* reported similar findings with a median splenic area of 59.2 cm^2 and diameter of 13.1cm in its cohort of 210 patients[19]. Given the excellent reproducibility seen in our study and confirmation of similar findings in one other study, spleen area may be a useful adjunct in predicting CSPH. Further research with an external cohort is needed to validate our findings."

Paragraph 5 of discussion (page 12): "Of note, the study by Giuffrè et al., which had a similar study population, demonstrated APRI to be a statistically significant determinant of CSPH with a similar median of 0.70[19]."

Paragraph 6 of discussion (page 13): "Finally, Giuffrè *et al.* was perhaps the most comparable of all the studies mentioned as his team used the ElastPQ model to develop the spleen stiffness probability index (SSPI) – a probability formula using spleen stiffness[19]."

Paragraph 7 of discussion [strengths and limitation] (page 13)): "Both the use of a novel pSWE machine (ElastPQ) and investigation of spleen area describe a unique approach in our study compared to others carried out in the field. To our knowledge, this is the one of the first studies to assess the role of the ElastPQ spleen stiffness, spleen area and splenic diameter measurements in predicting CSPH. As a result, our study took into consideration inter-operator variability of splenic area and diameter, which supported its potential use in clinical practice."

Paragraph 8 of the discussion [strengths and limitations] (page 14): "We did not exclude patients taking pharmacological treatment for PH from the original protocol as it was suspected that non-selective beta blockers and banding of varices would be unlikely to affect splenic measurements[32]. However, the most recent data on cirrhotic patients with high risk varices suggests that taking non-selective beta blockers can affect splenic stiffness[33, 34]. But these studies were undertaken using Fibroscan® and VTQ (Siemens Acuson S2000TM) ultrasound systems and so, further information is still needed in order to confirm that similar findings are present with the ElastPQ."

2. Also, in strengths and limitation: this is not the first study, but the one cited above. Authors should also discuss the time interval of one year between elastography and endoscopy.

Response: We have now amended the strengths and limitations sections of the study to reflect the comment above as found in paragraph 7 of discussion [strengths and limitations] (page 13): "To our knowledge, this is the one of the first studies to assess the role of the ElastPQ spleen stiffness, spleen area and splenic diameter measurements in predicting CSPH."