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Noninvasive diagnosis of portal hypertension in patients with compensated advanced chronic liver disease

--Manuscript Draft--

Manuscript Number:	AJG-20-1383R1
Full Title:	Noninvasive diagnosis of portal hypertension in patients with compensated advanced chronic liver disease
Article Type:	Article
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Abstract:	Objectives: We aimed to explore the prevalence of portal hypertension in the most common etiologies of patients with compensated advanced chronic liver disease (cACLD) and develop classification rules, based on liver stiffness measurement (LSM), that could be readily used to diagnose or exclude clinically significant portal hypertension (CSPH) in clinical practice. Methods: International cohort study including patients with paired LSM/hepatic venous pressure gradient (HVPG), LSM ≥10kPa and no prior decompensation. Portal hypertension was defined by an HVPG>5 mmHg. A positive predictive value (PPV) ≥90% was considered to validate LSM cut-offs for CSPH (HVPG≥10 mmHg), while a negative predictive value ≥90% ruled out CSPH. Results: 836 patients were evaluated: hepatitis C (HCV, n=358), non-alcoholic steatohepatitis (NASH, n=248), alcohol (ALD, n=203) and hepatitis B (HBV, n=27). Portal hypertension prevalence was >90% in all cACLD etiologies, except for NASH patients (60.9%), being even lower in NASH obese patients (53.3%); these lower prevalences of portal hypertension in NASH patients were maintained across different strata of LSM values. LSM≥25 kPa was the best cut-off to rule in CSPH in ALD, HBV, HCV and non-obese NASH patients, while in obese NASH patients the PPV was only 62.8%. A new model for NASH patients (ANTICIPATE-NASH model) to predict CSPH considering BMI, LSM and platelet count was developed and a nomogram constructed. LSM≤15 kPa plus platelets ≥150x10 9 /L ruled out CSPH in most etiologies. Conclusions: Patients with cACLD of NASH etiology, especially obese NASH patients, present lower prevalences of portal hypertension compared to other cACLD etiologies. LSM≥25 kPa is sufficient to rule in CSPH in most etiologies, including non-obese NASH patients, but not in obese NASH patients.
Response to Reviewers:	Point-by-point answers to reviewers' comments: Editor/Editorial Board Comments: Editors: After reviewing the manuscript and the comments from the peer reviewers, we would like to ask the authors to address the following issues raised by the editors: 1. Was there any correlation of their findings with EGD, and specifically with the absence/presence of high-risk esophageal varices warranting prophylaxis with beta-blockers or endoscopic band ligation? This a very good point, but information regarding endoscopy was not requested to the participating centers (only LSM and HVPG were available) and it was not an objective of this study. Only the “ANTICIPATE” cohort had endoscopy data and this has been published in the “Anticipate” paper (Hepatology 2016; 64:2173–84) and the Expanded Baveno paper (Hepatology 2017; 66:1980-8). 2. Can the authors please convert lab values in table 1 from SI to conventional units? Done. 3. We request the authors provide clearer instructions on how to use the nomogram from figure 3. Maybe they can provide an example or improve the instructions, something similar to what was described for the nomogram in figure 4 of the Hepatology 2016 paper on the "Anticipate" study. Thank you for the request. We have now changed the legend of figure 3 improving the instructions to follow the nomogram (similar to the”Anticipate” study) and providing an example to make more comprehensible the calculation. 4. We acknowledge and appreciate the authors sharing the fact they had submitted the manuscript to another journal first, and especially for providing the comments from

reviewers and the authors revisions. We think the quality of manuscript was improved by such revisions.

Thank you.

Reviewer #1: The authors present an excellent study which analyzed very interesting cohorts und revealed important insight especially in the emerging etiology NASH.
the two major findings in this paper on my opinion are:
1. the relationship of BMI with HVPG in NASH
2. a nomogram for the diagnosis of CSPH
the data support the conclusions and I have few comments:

1. alcoholic liver disease seems to have a more advanced disease. does this correlate also with the degree of fibrosis? in other words, are the groups also controlled for progression of disease?
As the reviewer mentions fibrosis degree is an important component that should be considered. Alcoholic patients, despite common inclusion criteria among groups, had higher mean LSM and HVPG, and higher prevalence of PH and CSPH compared to any other etiologic group. On the other hand, when analyzing the prevalence of PH in different ranges of LSM values (Suppl Table 1), alcoholic patients presented always a higher prevalence of PH compared to other etiologies. It seems that alcoholic patients have more advanced disease even with similar LSM values. As the reviewer points out, having information regarding liver histology, in addition to LSM and HVPG, could have helped understanding whether alcoholics presented a more advanced disease or at least, higher amounts of collagen deposition. As explained, this was not the aim of our study and liver histology was not available from these patients.
2. what about the treatments the patients have received or receive? do the authors see any relationship to NSBB, statins, ACE-I, or others?
This is another important variable that might have influenced HVPH values and that it was not taken into account when collecting the data and consequently, it cannot be analyzed. A comment has been added in the study limitations regarding this issue (pag. 14, last para.)
3. what was the time between LSM and HVPG?
It was 3 months (first para., Study cohorts section of Patient and Methods).
4. HCV patients show higher AST/ALT, yet LSM and HVPG are similar, do the authors see any effect of hepatic inflammation on LSM?
All HCV patients recruited had AST/ALT values below 200 U/L and as the reviewer mentions LSM and HVPG values of HCV patients were not much different from other groups (except for alcoholic patients who had higher values). We feel that the influence of AST/ALT values on LSM values of HCV values was very little.

Reviewer #2: Overall a well written and conceptualized paper.

This is an important clinical area as identifying the cohort of Child Pugh A patients with CSPH is important for their ongoing management.

I feel the discussion could explore in more detail the potential mechanisms for your finding of reduced CSPH in the nash cohort, especially as their bmi increases. Are there any other factors at play beyond the equipment i.e. M prob or XL probe?
The reason for the lower prevalence of PH and CSPH in the obese NASH population, despite similar LSM values to other etiologies, is unknown. From the theoretical point of view this could be caused by an overestimation of LSM by elastography in these patients (with similar liver damage) or because obese NASH patient livers produce less increments of portal pressure (with similar liver damage). Basically, two potential factors have been implicated for the first cause; the presence of liver fat and the presence of body fat. Both might be considered “technical issues” regarding interferences with the LSM readings by fat accumulation. Regarding liver fat, results are contradictory and recent studies point out to no or little influence. In relation to body fat and consequently abdominal girth, there are evidences that they might increase LSM readings. This has been discussed in our paper. LSM inaccuracy in NASH patients might be just a “technical issue” in obese people related to skin-to-liver distance or other physical characteristics or related to an interference in LSM readings caused by fat accumulation in the subcutaneous tissue. For the second mechanism

	(obese NASH patient livers causing less increments of portal pressure), without a very careful study with simultaneous liver biopsy (with collagen proportional area measurements), HVPG and LSM from NASH patients (obese and non-obese) it will be very difficult to have a convincing answer. In summary, liver biopsy is an important missing piece of this puzzle, and we have now acknowledged this limitation in our work in the Discussion (pag.14, end of 3rd para.).
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Brennan Spiegel, MD, Editor-in-Chief
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Dear Editors,

Thank you very much for the review and for considering a revised version of our paper addressing the minor concerns.

Enclosed please find a revised version with changes and a point-to-point response to the comments raised by the reviewers and editorial board.

We hope you find our paper worth of publication in *American Journal of Gastroenterology.*

Yours sincerely,

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Noninvasive diagnosis of portal hypertension in patients with compensated advanced chronic liver disease

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Conflict of Interests:

MMM served as advisory board member for Abbvie, Gilead, Merck, Novartis and Pfizer and received research/clinical trials support from Abbvie, Gilead, Siliagen and Transgene. MM served as a speaker and/or consultant and/or advisory board member for AbbVie, Bristol-Myers Squibb, Gilead, and W. L. Gore & Associates and received travel support from AbbVie, Bristol-Myers Squibb, and Gilead. PS received

speaker fees from Boehringer Ingelheim and Roche as well as travel support from Boehringer Ingelheim, Gilead and Roche. AF served as speaker and consultant for AbbVie and Gilead. TR served as a speaker and/or consultant and/or advisory board member for AbbVie, Bayer, Boehringer Ingelheim, Gilead, Intercept, MSD, Roche, Siemens, and W. L. Gore & Associates and received grants/research support from AbbVie, Boehringer Ingelheim, Gilead, MSD, Philips, and W. L. Gore & Associates as well as travel support from Boehringer Ingelheim, Gilead, and Roche. JGA consulting for Gilead, Pfizer, Genfit; has received lecture fees from Gilead, Ferring, Lupin and grant support from Gilead. MP, SA, BS, MG, MR, SGR, HS, MMF, BP, GS, AB, ET, CB, JB and JG have nothing to disclose.

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Authors contributions:

Study concept and design: SA, JGA, JG

Acquisition of the data: All authors

Analysis and interpretation of the data: MP, SA, JGA, JG

Drafting of the manuscript: MP, SA, JGA, JG

Critical review of the manuscript: All authors

All authors have reviewed and approved the manuscript

Study highlights:

What is known:

- Noninvasive tools can detect portal hypertension

- Prevalence of portal hypertension in compensated advanced chronic liver disease (cACLD) patients is unknown
- Valid liver stiffness cut-offs for identifying clinically significant portal hypertension (CSPH) across different cACLD etiologies are lacking

What is new here:

- The majority of cACLD patients present portal hypertension
- Non-alcoholic steatohepatitis (NASH) cACLD patients, especially obese, show a much lower prevalence of portal hypertension
- Liver stiffness ≥25 kPa is sufficient to rule in CSPH in cACLD patients, except in obese NASH patients.

Abbreviations:

cACLD: Compensated advanced chronic liver disease

LSM: Liver stiffness measurement

CSPH: Clinically significant portal hypertension

HVPG: Hepatic venous pressure gradient

PPV: Positive predictive value

NPV: Negative predictive value

HCV: Chronic hepatitis C

NASH: Non-alcoholic steatohepatitis

ALD: Alcoholic liver disease

HBV: Chronic hepatitis B

CI: Confidence interval

BMI: Body mass index

Key words:

Portal hypertension

Noninvasive tools

Liver stiffness

Compensated advanced chronic liver disease

Clinically significant portal hypertension

ABSTRACT

Objectives: We aimed to explore the prevalence of portal hypertension in the most common etiologies of patients with compensated advanced chronic liver disease (cACLD) and develop classification rules, based on liver stiffness measurement (LSM), that could be readily used to diagnose or exclude clinically significant portal hypertension (CSPH) in clinical practice. **Methods:** International cohort study including patients with paired LSM/hepatic venous pressure gradient (HVPG), LSM ≥10kPa and no prior decompensation. Portal hypertension was defined by an HVPG>5 mmHg. A positive predictive value (PPV) ≥90% was considered to validate LSM cut-offs for CSPH (HVPG≥10 mmHg), while a negative predictive value ≥90% ruled out CSPH. **Results:** 836 patients were evaluated: hepatitis C (HCV, n=358), non-alcoholic steatohepatitis (NASH, n=248), alcohol (ALD, n=203) and hepatitis B (HBV, n=27). Portal hypertension prevalence was >90% in all cACLD etiologies, except for NASH patients (60.9%), being even lower in NASH obese patients (53.3%); these lower prevalences of portal hypertension in NASH patients were maintained across different strata of LSM values. LSM≥25 kPa was the best cut-off to rule in CSPH in ALD, HBV, HCV and non-obese NASH patients, while in obese NASH patients the PPV was only 62.8%. A new model for NASH patients (ANTICIPATE-NASH model) to predict CSPH considering BMI, LSM and platelet count was developed and a nomogram constructed. LSM≤15 kPa plus platelets ≥150x10^9/L ruled out CSPH in most etiologies. **Conclusions:** Patients with cACLD of NASH etiology, especially obese NASH patients, present lower prevalences of portal hypertension compared to other cACLD etiologies. LSM≥25 kPa is sufficient to rule in CSPH in most etiologies, including non-obese NASH patients, but not in obese NASH patients.

Introduction

In 2015, Baveno VI consensus introduced new concepts, based on noninvasive methods, regarding the diagnosis and management of advanced chronic liver disease, reflecting the widespread use of these methods, especially transient elastography, in clinical practice for liver disease staging [1]. At the same time, there has been an increasing interest in identifying noninvasive methods to detect the presence of clinically significant portal hypertension (CSPH) and varices, two of the main hallmarks in cirrhosis, in order to avoid the invasiveness of hepatic venous pressure gradient (HVPG) and reduce the number of screening upper endoscopies needed in these patients.

In asymptomatic patients with chronic liver disease, due to the difficulty to distinguish severe fibrosis from cirrhosis, the new term "compensated advanced chronic liver disease (cACLD)" was introduced to define never decompensated patients with chronic liver disease detected by noninvasive methods at risk of developing CSPH. In patients with known causes of chronic liver disease, liver stiffness measurements (LSM) <10 kPa rule out cACLD and values >15 kPa are highly suggestive of cACLD [1]. However, the prevalence of portal hypertension in the different etiologies of cACLD patients is not currently known.

Another new concept at Baveno VI was that in viral-related cACLD, noninvasive methods were enough to rule in CSPH when LSM, alone or combined to platelet count and spleen size, was ≥20-25 kPa. These cut-offs were based on studies showing a high specificity and positive predictive values (PPV) in detecting CSPH [2–4]. It is important to keep in mind that for HCV patients the proposed criteria were referred to untreated patients, since assessing CSPH with LSM in HCV cured patients is much more difficult. However, based on this recommendation, it is not clear which is the cut-off of LSM that is most accurate to predict the risk of having CSPH, if and when platelets or spleen size are needed to improve risk assessment, and whether the same classification rule could be applied to non-viral etiologies, especially in the currently growing population of non-alcoholic steatohepatitis (NASH) patients. In addition, in the recently published ANTICIPATE study, noninvasive tests (including LSM and platelets) were also used for a continuous risk prediction model to individualize the risk of having CSPH [5], but this strategy has not been validated in other cohorts, especially in NASH patients. Finally, the recent publication of the results of the PREDESCI study indicating that β-blockers prevent hepatic decompensation in patients with CSPH [6], clearly reinforce the relevance of having noninvasive tools for detecting these patients.

The aims of the present study were: 1) to analyze the prevalence of portal hypertension in the main etiologies of cACLD; 2) to validate the ANTICIPATE models for CSPH; and 3) to develop simple classification rules based on LSM to diagnose or exclude CSPH in the main etiologies of cACLD.

Patients and methods

Study cohorts

Data from different international cohorts including patients of different etiologies of chronic liver disease were analyzed. Inclusion criteria were the same for all cohorts; all patients had LSM ≥10 kPa (suggestive of cACLD), normal liver function (equivalent to Child-Pugh class A) and no prior decompensation. In addition, patients should not have been on β-blocker therapy for varices and paired data on LSM and HVPG should have been obtained within 3 months. Patients with infrequent etiologies, in addition to underrepresented etiologies within the whole set of data were also excluded.

The present study comprises the analysis of two different types of cohorts: a re-analysis of retrospective data from previously published databases along with analysis of newly collected data. Regarding retrospective data, two large cohorts were analyzed. In the ANTICIPATE original cohort [5,7], 542 patients from four centers in Europe (one in France, one in Romania, and two in Spain) and one in Canada were evaluated for noninvasive prediction of portal hypertension. Patients from the European centers were reported, in part, in previous publications; however, there were no data regarding the total number of patients evaluated before the inclusion [8–11]. In total, 216 patients with LSM and HVPG were available for the present study. The other retrospective cohort came from Vienna and included 400 patients meeting inclusion criteria out of a total of 1908 patients. These patients formed part of different studies from the Vienna group and were partially reported in previous publications [12–17]. Patients excluded for the present study from both cohorts are described in supplementary data.

In addition to these two large cohorts, we recruited 220 new additional patients, essentially cACLD NASH patients (170 of the 220; 77.2%), because this etiology was clearly underrepresented in the retrospective cohorts. The distribution of patients from different centers was the following: 74 from Toulouse, 50 from Vienna, 37 from Bern, 34 from London and 25 from Barcelona. These patients were recruited in each center as part of ongoing prospective NASH study cohorts during the period 2016-2018. HVPG and liver biopsy were performed in these patients either as part of routine clinical evaluation or to evaluate them for participating in NASH clinical trials. All liver biopsies from these patients were diagnostic of NASH, except for the cases with cirrhosis and absence of steatohepatitis, in which the etiology was attributed to NASH when the patient profile was compatible and other etiologies excluded. Information about fibrosis stage of the patients was not collected, since this was not the objective of the present study. Only patients meeting the above mentioned inclusion criteria were selected. Excluded patients are described in supplementary data.

HCV cACLD was diagnosed when anti-HCV and HCV-RNA were positive; none of the HCV patients had received antiviral treatment at the time of participating in this study. Alcoholic liver disease (ALD) was

defined by a history of chronic alcohol consumption of ≥40 g per day in men and ≥30 g in women. NASH diagnosis was based in the histological presence of steatohepatitis, excluding other causes of fat liver accumulation, including alcohol consumption.

Main outcomes

The outcomes under study were: 1) Presence of portal hypertension defined by an HVPG of 6 mmHg or more, and 2) CSPH defined as an HVPG of 10 mmHg or more. For CSPH the objective was to find simple classification rules using noninvasive tests, including LSM, to rule in and out CSPH in the different etiologies. For ruling in a ≥90% PPV was accepted, while for ruling out it was a ≥90% negative predictive value (NPV).

Transient elastography

For the two retrospective cohorts transient elastography by Fibroscan (Echosens, Paris, France) was utilized to obtain LSM. The quality criteria used for LSM were the criteria recommended at the time of the inclusion of the cohorts: 10 valid measurements obtained with a success rate ≥60% and the interquartile to median ratio (IQR/M) ≤30%. An M probe was used in all measurements. Data of the number of unreliable/invalid LSM excluded are not available from the ANTICIPATE cohort. It is worth to mention that 22.8% of NASH patients included in the study were evaluated with an M probe, without the possibility of using the XL probe if needed.

By contrast, the newest cohorts during 2016-2018, including the majority of NASH patients, used a newer version of Fibroscan (Fibroscan 502 Touch) with availability of M and XL probes in all centers, that were used based on device requirements. Quality criteria used in all centers were: at least 10 valid measurements and an IQR/M ≤30%.

Hepatic venous pressure gradient

HVPG was performed with the standard balloon-catheter technique by experienced personnel [18]. All centers participating in the study have large experience in HVPG studies and many publications from the different authors describe the technique.

Statistics

Numerical variables were described as median (interquartile range), and categorical variables as absolute and relative frequencies. For classification rules, PPV/NNP were prioritized to sensitivity/specificity because our aim was to find subpopulations with high and low prevalences of CSPH. In addition, we tried

to develop classification rules as simple as possible in order to facilitate clinical application by using the lowest possible number of parameters to elaborate the rule, always prioritizing LSM. Calibration of the ANTICIPATE models in the new sample was tested by plotting observed vs predicted frequencies of CSPH, excluding all patients already belonging to the ANTICIPATE cohort [19]. The association between LSM, BMI and CSPH in patients with NASH was modeled with logistic regression from which the nomogram to estimate the probability of CSPH was derived (detailed methods are provided as supplementary data). The association between BMI, LSM and platelet count with HVPG values was explored with linear regression (detailed methods are provided as supplementary data). Analysis was conducted with STATA 13.1 statistical software (StataCorp, College Station, TX, US) and with R Statistical Software (Foundation for Statistical Computing, Vienna, Austria), using the rms (Frank E Harrell Jr (2018). rms: Regression Modeling Strategies. R package version 5.1-2. https://CRAN.R-project.org/package=rms) and ggplot2 (H. Wickham. ggplot2: Elegant Graphics for Data Analysis. Springer-Verlag New York, 2016) packages.

Results

A total of 836 patients were included in the study. Of them, 358 patients (42.8%) had HCV, 248 (29.7%) NASH, 203 (24.3%) ALD, and 27 patients (3.2%) chronic hepatitis B (HBV). Table 1 shows the baseline characteristics of the different cohorts. Most of the patients were male and had a normal liver function equivalent to Child Pugh score of 5. The prevalence of portal hypertension (HVPG >5 mmHg) in the whole cohort was 83%, while the prevalence of CSPH was 59%.

Differences in baseline characteristics were observed in some etiologies. ALD patients had higher LSM values and a higher prevalence of portal hypertension and CSPH compared to other etiologies. NASH patients had, as expected, a higher body mass index (BMI) than other etiologies, were significantly older and presented higher platelet count. The median HVPG in NASH patients was 7 mmHg, lower than in other etiologies, being the prevalence of portal hypertension and CSPH in NASH patients the lowest.

Prevalence of portal hypertension (HVPG >5 mmHg)

As explained, the prevalence of portal hypertension was different across the distinct etiologies of cACLD patients (Table 1 and 2). HCV and ALD patients were not much different, but NASH patients presented a remarkably lower prevalence of portal hypertension that was maintained across different strata of LSM values (Table 2 and suppl. table 1).

Supplementary table 2 compares the clinical characteristics between NASH patients with and without portal hypertension. As seen, NASH patients without portal hypertension had higher BMI compared to

those with portal hypertension. As seen in table 2, stratifying NASH patients according to BMI showed a much lower prevalence of portal hypertension in all subgroups of obese (BMI ≥30 kg/m^2) patients. In addition, this low prevalence of portal hypertension in obese NASH patients was not due to a lack of use of XL probe for obese patients, since when the analysis was restricted to the NASH patient cohorts with availability of XL probe, portal hypertension prevalence was even lower (Table 2).

Detecting CSPH (HVPG ≥10 mmHg)

Prevalence of CSPH (59%) was different between etiologies of cACLD (Table 1 and figure 1); ALD had the highest prevalence (83.3%) and NASH patients the lowest (39.1%). However, in NASH patients, the prevalence of CSPH changed according to BMI, decreasing the prevalence as BMI increased. The prevalence of CSPH in NASH patients with BMI <30 kg/m^2 was 50.5% (51/101) and 30.8% (41/133) in patients with BMI ≥30 kg/m^2 (Figure 1).

Validation of ANTICIPATE models

The performance of two previously described models from the ANTICIPATE study for risk prediction of CSPH, one using LSM and one using LSM plus platelet count was tested for different etiologies. Those patients already included in the ANTICIPATE study were excluded for this analysis. ALD and HCV patients (etiologies reflecting the composition of the sample in the original ANTICIPATE cohort) were analyzed together, since results were not different when assessed individually. Both models predicted CSPH, demonstrating a very good calibration in the subsample of patients with ALD/HCV etiology (Supplementary figures 1A and 1B). In supplementary figure 2, we illustrate the pointwise predicted risk of individual LSM values by the ANTICIPATE model and the observed accumulated prevalence of CSPH in different ranges of LSM and above a defined value of LSM in ALD plus HCV patients. As shown in the figure, predicted and observed CSPH prevalences increase with increasing LSM values.

In contrast to patients with ALD and HCV, in patients with NASH cirrhosis both models were substantially miscalibrated, considerably overpredicting the risk of CSPH (supplementary figures 1C and 1D).

LSM cut-off for ruling in CSPH

The whole cohort of patients in the study was used to explore the best LSM cut-off for ruling in CSPH with a PPV ≥90%. The accumulated prevalences of CSPH were 83.5% for LSM ≥20 kPa, 91% for LSM ≥25 kPa and 93.7% for LSM ≥30 kPa. Therefore, LSM ≥25 kPa was chosen as the optimal cut-off for ruling in CSPH in our cohort.

When assessing different etiologies, this cut-off performed well for most of the etiologies. There were 144/155 ALD patients (96.6%) with LSM ≥25 kPa and CSPH, 120/130 HCV patients (92.3%) and 11/11 (100%) HBV patients (Figure 1). However, in keeping with the data shown in supplementary figure 1, in NASH patients this cut-off did not provide the pre-specified 90% PPV target; only 57 out of 74 NASH patients (77%) had CSPH above this LSM cut-off. Additional information regarding the performance of the LSM ≥25 kPa for ruling in CSPH is shown in supplementary table 3.

CSPH prediction in NASH patients

Supplementary table 4 shows the differences between NASH patients with LSM ≥25 kPa and CSPH compared to patients without CSPH. Again, one important difference between those groups was BMI. The prevalence of CSPH was higher in non-obese patients with 33/36 patients (91.7%) with LSM ≥25 kPa showing CSPH, while obese patients had lower prevalence of CSPH despite high LSM values (22/35, 62.8%) (Figure 1). Figure 2 represents the predicted probability of CSPH by logistic regression model according to the presence of obesity (BMI ≥30 kg/m^2 vs BMI <30 kg/m^2) (Figure 2A), and according to different BMI values (Figure 2B). For obese NASH patients, the figure shows that the prevalence of CSPH was always <90% whatever the LSM chosen. By contrast, in etiologies other than NASH, BMI did not seem to impact in the effect of LSM to predict the risk of CSPH (Supplementary figure 3).

Since predictions of the ANTICIPATE model were suboptimal in this series and BMI impacted non-invasive predictions of CSPH, we developed a modified nomogram to predict CSPH on the basis of LSM, BMI and platelet count (Figure 3 and supplementary data). Both discrimination (c-statistic 0.90) and calibration (graphically shown in supplementary data) of this refined model were excellent in the present sample.

Association between LSM, Platelet count and HVPG according to BMI in NASH patients

Figure 4 shows the influence of BMI on the non-invasive prediction of HVPG either with LSM alone (Figure 4A) or LSM+platelet count (Figure 4B) in NASH patients. For a given LSM value or a combination of LSM+platelet count values, HVPG was lower for higher BMIs. This trend is also shown (with further granularity) in supplementary figure 4.

<u>Ruling out CSPH: High NPV ≥90%</u>

In the ANTICIPATE model it was not possible to find a population with a very low risk (<10%) of having CSPH. In this cohort, with LSM alone it was also not possible to identify a cut-off with high NPV to exclude CSPH, although in NASH patients the LSM <13.6 kPa had the highest NPV (Table 3). However, adding platelet count ≥150x10^9/L to a LSM ≤15 kPa cut-off could reasonably exclude CSPH in most etiologies

(except for HBV that included a very low number of patients). Additional information regarding the performance of the proposed parameters for ruling out CSPH is shown in supplementary table 3.

Discussion

The present study demonstrates that LSM alone is useful to select patients with chronic liver disease at risk of having portal hypertension and CSPH for most etiologies, including ALD, HCV and HBV. At the same time, our work illustrates that in NASH patients, and especially obese NASH patients, these LSM-based rules behave differently from other etiologies, in part because the association between LSM and HVPG changes with BMI in patients with NASH. It is important to keep in mind that our aim was to analyze the relationship between LSM and HVPG values in populations that were selected based on LSM values and not on histological staging or clinical features.

The first important hallmark of our study was the differences in portal hypertension prevalence among patients with cACLD from different etiologies, selected on the basis of a LSM 10 kPa. Patients with HCV, ALD and HBV presented prevalences above 90% that increased to nearly 100% when subgroups with higher LSM values were selected. By contrast, NASH patients had a lower portal hypertension prevalence in the whole population and also in the different subgroups determined by higher LSM values. Obesity seemed to be, in part, responsible for the lower prevalence of portal hypertension, since obese patients (BMI ≥30 kg/m^2) showed even lower rates of portal hypertension as compared to other etiologies and nonobese NASH patients. Recent studies have suggested that obesity can reduce the diagnostic accuracy of LSM for advanced liver fibrosis in NASH patients by increasing the rates of false positives; this worse diagnostic performance is also seen when M or XL probes are used [20]. In addition, Wong, et al [21] have shown that obesity might be responsible for higher LSM values as compared to nonobese NASH patients with the same fibrosis stage. A similar situation might be taking place in our study, generating lower rates of portal hypertension in NASH patients as compared to other etiologies within the same LSM values strata. Also, in our study, the availability of the XL probe for the prospective cohort of NASH patients did not resulted in an increased prevalence of portal hypertension, but rather the opposite.

Another objective of our study was to develop classification rules for diagnosing and excluding CSPH in the different etiologies of cACLD patients that could be readily used in clinical practice. Baveno VI recommendations were restricted to viral etiologies and the proposed rules were not clearly defined [1]. Our data indicate that LSM ≥25 kPa is sufficient to rule in CSPH in ALD, HBV, HCV and nonobese NASH patients with PPV higher than 90%, without the need of additional noninvasive parameters. In terms of applicability, having a single parameter with a single cut-off will undoubtedly facilitate the point-of-care

use of the classification rule by clinicians. Considering that prophylactic measures to prevent cirrhosis decompensation, such as β-blockers, might be established as standard of care in patients with CSPH in the future [6], the simple rules presented here would clearly help in patient selection. In addition, the ANTICIPATE model for predicting CSPH also showed a very good calibration in the population of ALD and HCV patients, indicating that it is a robust model when applied to populations with etiologies similar to the sample where it was derived. This model could also be utilized to select patients at risk of CSPH, especially patients with LSM between 15 and 25 kPa.

Again, a very different scenario was found in NASH patients, especially obese NASH patients. Taken together, the information regarding NASH patients can be summarized as follows: 1) the prevalence of CSPH in the NASH population with LSM ≥25 kPa is lower (77%) than in other etiologies resulting in an insufficient PPV; 2) obese NASH patients (BMI ≥30 kg/m^2) with LSM ≥25 kPa are the subgroup with the lowest prevalence of CSPH (62.8%); 3) the ANTICIPATE model overpredicts the presence of CSPH in these patients (Supplementary figure 1C and D); 4) obesity seems to influence LSM prediction of CSPH only in NASH patients (Figure 2A and B), as opposed to obese non-NASH patients (supplementary figure 3); 5) the impact of BMI on LSM predictions of CSPH increases with higher BMIs (Figure 2B); and 6) Increasing LSM cut-off did not improve sufficiently CSPH prediction in NASH patients. As an alternative, a new model for NASH patients (ANTICIPATE-NASH model) to predict CSPH was developed using LSM, platelet count and BMI, and a nomogram for practical use constructed.

One issue that is relevant for interpreting the data presented here refers to the basal differences between the distinct etiologic groups of patients, and especially the NASH group from the others. In this study, using the same inclusion criteria in all patients, NASH patients were different in terms of prevalence of portal hypertension and CSPH, having the lowest prevalence of the total cohort. It could be argued that this might have affected the results in terms of prediction; the lowest the prevalence of a feature, the lowest the probability of having high PPV. The different cohorts evaluated here are a reflection of the type of patients currently seen in tertiary hospitals. For the case of NASH patients, many of them are probably referred from primary care centers for suspicion of advanced disease and evaluated for entering NASH clinical trials, selecting a less sick population and consequently introducing a selection bias in the analysis. However, it is not less true that all patients were included based on an objective cut-off LSM value and the percentage of LSM <20 kPa in NASH patients was not different from HCV and HVB patients. Also, as explained before, the differences in prevalence of portal hypertension and CSPH observed in NASH patients were preserved in all sub-strata of LSM values, excluding a selection bias of the recruited population as the main cause for this difference. Thus, the issue is why NASH patients with the same LSM values (or range of LSM values) showed less portal hypertension and CSPH than other etiologies or in

other words, what lies behind the observation that obese NASH patients have less severe liver disease, despite similar LSM values. This cannot be answered in the present study and many other reports have given contradictory results regarding the effect of body and liver fat in LSM readings [20,22–25]. In a study by Petta, et al. performed in 253 patients with NASH and using only M probe, they found that in patients with low fibrosis (F0 to F2) median values of LSM were higher in those patients with severe steatosis at liver biopsy compared to those without, increasing the rates of false positive LSM values [23]. Similar data was obtained later on in a study with 2058 patients with mixed etiologies (18% NASH) where they found a slight effect of steatosis in increasing LSM values in patients with absent or mild fibrosis, but not in more advanced stages of fibrosis [24]. However, recently, a multicentric study including 450 NASH patients with paired liver biopsy and LSM (using M or XL probes as needed) found that the only parameter affecting LSM values was fibrosis stage and no association was found with steatosis or probe type [22]. None of these previous studies evaluated BMI as a possible factor that could influence LSM values. Wong, et al. found that obese NASH patients (BMI ≥30 kg/m^2) had higher LSM than those with BMI <30 kg/m^2 at all fibrosis stages in liver biopsy and for both M and XL probes [21]. Myers, et al. evaluated factors influencing discordance in fibrosis staging using XL probe and they found that the only factor associated with fibrosis staging discordance between liver biopsy and LSM was BMI, overestimating liver fibrosis measured by LSM [25]; patients with BMI ≥40 kg/m^2 and LSM ≥7 kPa had the highest rates of discordance. Therefore, it remains unclear whether LSM inaccuracy in NASH patients is just a "technical issue" in obese people related to skin-to-liver distance or other physical characteristics, or if fat might increase liver stiffness without fibrosis. Liver biopsy information from our patients, unfortunately unavailable, might have shed some light in clarifying these possible mechanisms. What it is very clear is that overall in NASH patients, LSM is not a good predictor of advanced fibrosis and cirrhosis with very low PPV and many different cut-offs proposed [23,26]; this inconsistency is probably reflected here when predicting CSPH in these patients.

Finally, in terms of excluding CSPH in our cohort with a NPV of ≥90%, we observed that LSM ≤15 kPa plus platelet count ≥150x10^9/L was useful for ruling out CSPH in the whole cohort and in all etiologies. However, in HBV, the number of patients was too low to confirm the rule.

Our study has some limitations. The two main cohorts were retrospective and mainly composed of HCV and ALD patients, lacking the XL probe in all LSM performed. The newer ones enriched with NASH patients were not specifically recruited for the present study and not all NASH patients could have the possibility of using the XL probe. Using LSM by transient elastography as the main non-invasive parameter is a limitation for countries or centers with no availability for the technique. Also, concomitant treatments that might have influenced HVPG values (β-blockers, statins and others) have not been considered in our

study. On the other hand, differences in prevalences of portal hypertension or CSPH could have influenced the results of PPV or NPV. However, as mentioned before, this cohort is a reflection of the patients we currently see in our tertiary hospitals. We also acknowledge that selecting patients, mainly NASH patients, with a LSM ≥10 kPa might have prevented detecting patients with portal hypertension and no or mild fibrosis, as it has been reported in a few studies (27,28). Finally, we acknowledge the need of future validation of the NASH patient data, including the lowest prevalences of portal hypertension and CSPH, and the utility of the proposed ANTICIPATE-NASH patient model.

The strengths of the present study are that this is a large multicentric cohort including a large number of patients with the main etiologies of chronic liver disease (especially the new data on NASH patients), increasing the external validity of its results. Our classification rules are simple rules that can be applied in daily clinical practice without the need of complex calculations. Figure 5 summarizes the different LSM cut-offs indicative of cACLD and CSPH.

In conclusion, the present study shows that in patients with cACLD (defined as a LSM >10 kPa) portal hypertension is present in more than 90% when the etiology is ALD, HCV and HBV, whereas in NASH patients with cACLD, and especially in those with obesity, prevalence of portal hypertension is much lower. Regarding the non-invasive estimation of CSPH, Baveno VI LSM ≥25 kPa cut-off is sufficient to rule in CSPH in ALD, HBV, HCV and nonobese NASH patients, but is not specific enough in obese NASH patients. Finally, LSM ≤15 kPa plus platelet count ≥150x10^9/L can rule out CSPH in most etiologies of chronic liver disease.

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Figure legends

Figure 1. Prevalence of clinically significant portal hypertension (CSPH) by etiologies including all patients in each group (in blue) and only patients with liver stiffness measurement (LSM) ≥25 kPa (in red). Non obese NASH: BMI <30 kg/m^2; obese NASH: BMI ≥30 kg/m^2.

Figure 2. Predicted probability by a logistic regression model of CSPH in NASH patients according to: A) the presence of obesity (body mass index (BMI) ≥30 kg/m^2 in red color line) or not (BMI <30 kg/m^2 in blue color line); B) different BMI.

Figure 3. Nomogram to predict the presence of clinically significant portal hypertension (CSPH) in patients with non-alcoholic steatohepatitis (NASH) using the variables liver stiffness measurement (LSM), body mass index (BMI) and platelet count. To obtain the risk of CSPH trace a vertical line from each of the three predictors' axis to the first line ("points"). Add the total points and trace a vertical line from the "total points" axis to the probability axis to calculate the risk of CSPH. As shown, a patient with a LSM value of 20 kPa (29 points), a BMI of 35 (9 points) and a platelet count of 150x10^9 (67 points) would have a predictive probability of CSPH of 40% (for a total of 105 points).

Figure 4. Influence of body mass index (BMI) on the predicted values of hepatic venous pressure gradient (HVPG), based on liver stiffness measurement (LSM) alone or with LSM+platelets in NASH patients. As shown in panel A, for any given value of LSM, mean HVPG values are lower for higher BMIs. Panel B shows a similar observation. For any given combination of LSM and platelet count, predicted mean HVPG is lower as BMI increases. These figures are the graphical representation of the linear regression models outlined in the supplementary data.

Figure 5. The "rule of five". Proposed liver stiffness measurement cut-offs for ruling in and out compensated advanced chronic liver disease (cACLD) and clinically significant portal hypertension (CSPH), in addition to the Baveno criteria for avoiding screening endoscopy. Information was extracted from data from this study and the Baveno VI recommendations [1]. Plat: platelet count; HCV: Chronic hepatitis C; HBV: Chronic hepatitis B; ALD: Alcoholic liver disease; NASH: Non-alcoholic steatohepatitis

Noninvasive diagnosis of portal hypertension in patients with compensated advanced chronic liver disease

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Conflict of Interests:

MMM served as advisory board member for Abbvie, Gilead, Merck, Novartis and Pfizer and received research/clinical trials support from Abbvie, Gilead, Siliagen and Transgene. MM served as a speaker and/or consultant and/or advisory board member for AbbVie, Bristol-Myers Squibb, Gilead, and W. L. Gore & Associates and received travel support from AbbVie, Bristol-Myers Squibb, and Gilead. PS received

speaker fees from Boehringer Ingelheim and Roche as well as travel support from Boehringer Ingelheim, Gilead and Roche. AF served as speaker and consultant for AbbVie and Gilead. TR served as a speaker and/or consultant and/or advisory board member for AbbVie, Bayer, Boehringer Ingelheim, Gilead, Intercept, MSD, Roche, Siemens, and W. L. Gore & Associates and received grants/research support from AbbVie, Boehringer Ingelheim, Gilead, MSD, Philips, and W. L. Gore & Associates as well as travel support from Boehringer Ingelheim, Gilead, and Roche. JGA consulting for Gilead, Pfizer, Genfit; has received lecture fees from Gilead, Ferring, Lupin and grant support from Gilead. MP, SA, BS, MG, MR, SGR, HS, MMF, BP, GS, AB, ET, CB, JB and JG have nothing to disclose.

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Authors contributions:

Study concept and design: SA, JGA, JG

Acquisition of the data: All authors

Analysis and interpretation of the data: MP, SA, JGA, JG

Drafting of the manuscript: MP, SA, JGA, JG

Critical review of the manuscript: All authors

All authors have reviewed and approved the manuscript

Study highlights:

What is known:

- Noninvasive tools can detect portal hypertension

- Prevalence of portal hypertension in compensated advanced chronic liver disease (cACLD) patients is unknown
- Valid liver stiffness cut-offs for identifying clinically significant portal hypertension (CSPH) across different cACLD etiologies are lacking

What is new here:

- The majority of cACLD patients present portal hypertension
- Non-alcoholic steatohepatitis (NASH) cACLD patients, especially obese, show a much lower prevalence of portal hypertension
- Liver stiffness ≥25 kPa is sufficient to rule in CSPH in cACLD patients, except in obese NASH patients.

Abbreviations:

cACLD: Compensated advanced chronic liver disease

LSM: Liver stiffness measurement

CSPH: Clinically significant portal hypertension

HVPG: Hepatic venous pressure gradient

PPV: Positive predictive value

NPV: Negative predictive value

HCV: Chronic hepatitis C

NASH: Non-alcoholic steatohepatitis

ALD: Alcoholic liver disease

HBV: Chronic hepatitis B

CI: Confidence interval

BMI: Body mass index

Key words:

Portal hypertension

Noninvasive tools

Liver stiffness

Compensated advanced chronic liver disease

Clinically significant portal hypertension

ABSTRACT

Objectives: We aimed to explore the prevalence of portal hypertension in the most common etiologies of patients with compensated advanced chronic liver disease (cACLD) and develop classification rules, based on liver stiffness measurement (LSM), that could be readily used to diagnose or exclude clinically significant portal hypertension (CSPH) in clinical practice. **Methods:** International cohort study including patients with paired LSM/hepatic venous pressure gradient (HVPG), LSM ≥10kPa and no prior decompensation. Portal hypertension was defined by an HVPG>5 mmHg. A positive predictive value (PPV) ≥90% was considered to validate LSM cut-offs for CSPH (HVPG≥10 mmHg), while a negative predictive value ≥90% ruled out CSPH. **Results:** 836 patients were evaluated: hepatitis C (HCV, n=358), non-alcoholic steatohepatitis (NASH, n=248), alcohol (ALD, n=203) and hepatitis B (HBV, n=27). Portal hypertension prevalence was >90% in all cACLD etiologies, except for NASH patients (60.9%), being even lower in NASH obese patients (53.3%); these lower prevalences of portal hypertension in NASH patients were maintained across different strata of LSM values. LSM≥25 kPa was the best cut-off to rule in CSPH in ALD, HBV, HCV and non-obese NASH patients, while in obese NASH patients the PPV was only 62.8%. A new model for NASH patients (ANTICIPATE-NASH model) to predict CSPH considering BMI, LSM and platelet count was developed and a nomogram constructed. LSM≤15 kPa plus platelets ≥150x10^9/L ruled out CSPH in most etiologies. **Conclusions:** Patients with cACLD of NASH etiology, especially obese NASH patients, present lower prevalences of portal hypertension compared to other cACLD etiologies. LSM≥25 kPa is sufficient to rule in CSPH in most etiologies, including non-obese NASH patients, but not in obese NASH patients.

Introduction

In 2015, Baveno VI consensus introduced new concepts, based on noninvasive methods, regarding the diagnosis and management of advanced chronic liver disease, reflecting the widespread use of these methods, especially transient elastography, in clinical practice for liver disease staging [1]. At the same time, there has been an increasing interest in identifying noninvasive methods to detect the presence of clinically significant portal hypertension (CSPH) and varices, two of the main hallmarks in cirrhosis, in order to avoid the invasiveness of hepatic venous pressure gradient (HVPG) and reduce the number of screening upper endoscopies needed in these patients.

In asymptomatic patients with chronic liver disease, due to the difficulty to distinguish severe fibrosis from cirrhosis, the new term "compensated advanced chronic liver disease (cACLD)" was introduced to define never decompensated patients with chronic liver disease detected by noninvasive methods at risk of developing CSPH. In patients with known causes of chronic liver disease, liver stiffness measurements (LSM) <10 kPa rule out cACLD and values >15 kPa are highly suggestive of cACLD [1]. However, the prevalence of portal hypertension in the different etiologies of cACLD patients is not currently known.

Another new concept at Baveno VI was that in viral-related cACLD, noninvasive methods were enough to rule in CSPH when LSM, alone or combined to platelet count and spleen size, was ≥20-25 kPa. These cut-offs were based on studies showing a high specificity and positive predictive values (PPV) in detecting CSPH [2–4]. It is important to keep in mind that for HCV patients the proposed criteria were referred to untreated patients, since assessing CSPH with LSM in HCV cured patients is much more difficult. However, based on this recommendation, it is not clear which is the cut-off of LSM that is most accurate to predict the risk of having CSPH, if and when platelets or spleen size are needed to improve risk assessment, and whether the same classification rule could be applied to non-viral etiologies, especially in the currently growing population of non-alcoholic steatohepatitis (NASH) patients. In addition, in the recently published ANTICIPATE study, noninvasive tests (including LSM and platelets) were also used for a continuous risk prediction model to individualize the risk of having CSPH [5], but this strategy has not been validated in other cohorts, especially in NASH patients. Finally, the recent publication of the results of the PREDESCI study indicating that β-blockers prevent hepatic decompensation in patients with CSPH [6], clearly reinforce the relevance of having noninvasive tools for detecting these patients.

The aims of the present study were: 1) to analyze the prevalence of portal hypertension in the main etiologies of cACLD; 2) to validate the ANTICIPATE models for CSPH; and 3) to develop simple classification rules based on LSM to diagnose or exclude CSPH in the main etiologies of cACLD.

Patients and methods

Study cohorts

Data from different international cohorts including patients of different etiologies of chronic liver disease were analyzed. Inclusion criteria were the same for all cohorts; all patients had LSM ≥10 kPa (suggestive of cACLD), normal liver function (equivalent to Child-Pugh class A) and no prior decompensation. In addition, patients should not have been on β-blocker therapy for varices and paired data on LSM and HVPG should have been obtained within 3 months. Patients with infrequent etiologies, in addition to underrepresented etiologies within the whole set of data were also excluded.

The present study comprises the analysis of two different types of cohorts: a re-analysis of retrospective data from previously published databases along with analysis of newly collected data. Regarding retrospective data, two large cohorts were analyzed. In the ANTICIPATE original cohort [5,7], 542 patients from four centers in Europe (one in France, one in Romania, and two in Spain) and one in Canada were evaluated for noninvasive prediction of portal hypertension. Patients from the European centers were reported, in part, in previous publications; however, there were no data regarding the total number of patients evaluated before the inclusion [8–11]. In total, 216 patients with LSM and HVPG were available for the present study. The other retrospective cohort came from Vienna and included 400 patients meeting inclusion criteria out of a total of 1908 patients. These patients formed part of different studies from the Vienna group and were partially reported in previous publications [12–17]. Patients excluded for the present study from both cohorts are described in supplementary data.

In addition to these two large cohorts, we recruited 220 new additional patients, essentially cACLD NASH patients (170 of the 220; 77.2%), because this etiology was clearly underrepresented in the retrospective cohorts. The distribution of patients from different centers was the following: 74 from Toulouse, 50 from Vienna, 37 from Bern, 34 from London and 25 from Barcelona. These patients were recruited in each center as part of ongoing prospective NASH study cohorts during the period 2016-2018. HVPG and liver biopsy were performed in these patients either as part of routine clinical evaluation or to evaluate them for participating in NASH clinical trials. All liver biopsies from these patients were diagnostic of NASH, except for the cases with cirrhosis and absence of steatohepatitis, in which the etiology was attributed to NASH when the patient profile was compatible and other etiologies excluded. Information about fibrosis stage of the patients was not collected, since this was not the objective of the present study. Only patients meeting the above mentioned inclusion criteria were selected. Excluded patients are described in supplementary data.

HCV cACLD was diagnosed when anti-HCV and HCV-RNA were positive; none of the HCV patients had received antiviral treatment at the time of participating in this study. Alcoholic liver disease (ALD) was

defined by a history of chronic alcohol consumption of ≥40 g per day in men and ≥30 g in women. NASH diagnosis was based in the histological presence of steatohepatitis, excluding other causes of fat liver accumulation, including alcohol consumption.

Main outcomes

The outcomes under study were: 1) Presence of portal hypertension defined by an HVPG of 6 mmHg or more, and 2) CSPH defined as an HVPG of 10 mmHg or more. For CSPH the objective was to find simple classification rules using noninvasive tests, including LSM, to rule in and out CSPH in the different etiologies. For ruling in a ≥90% PPV was accepted, while for ruling out it was a ≥90% negative predictive value (NPV).

Transient elastography

For the two retrospective cohorts transient elastography by Fibroscan (Echosens, Paris, France) was utilized to obtain LSM. The quality criteria used for LSM were the criteria recommended at the time of the inclusion of the cohorts: 10 valid measurements obtained with a success rate ≥60% and the interquartile to median ratio (IQR/M) ≤30%. An M probe was used in all measurements. Data of the number of unreliable/invalid LSM excluded are not available from the ANTICIPATE cohort. It is worth to mention that 22.8% of NASH patients included in the study were evaluated with an M probe, without the possibility of using the XL probe if needed.

By contrast, the newest cohorts during 2016-2018, including the majority of NASH patients, used a newer version of Fibroscan (Fibroscan 502 Touch) with availability of M and XL probes in all centers, that were used based on device requirements. Quality criteria used in all centers were: at least 10 valid measurements and an IQR/M ≤30%.

Hepatic venous pressure gradient

HVPG was performed with the standard balloon-catheter technique by experienced personnel [18]. All centers participating in the study have large experience in HVPG studies and many publications from the different authors describe the technique.

Statistics

Numerical variables were described as median (interquartile range), and categorical variables as absolute and relative frequencies. For classification rules, PPV/NNP were prioritized to sensitivity/specificity because our aim was to find subpopulations with high and low prevalences of CSPH. In addition, we tried

to develop classification rules as simple as possible in order to facilitate clinical application by using the lowest possible number of parameters to elaborate the rule, always prioritizing LSM. Calibration of the ANTICIPATE models in the new sample was tested by plotting observed vs predicted frequencies of CSPH, excluding all patients already belonging to the ANTICIPATE cohort [19]. The association between LSM, BMI and CSPH in patients with NASH was modeled with logistic regression from which the nomogram to estimate the probability of CSPH was derived (detailed methods are provided as supplementary data). The association between BMI, LSM and platelet count with HVPG values was explored with linear regression (detailed methods are provided as supplementary data). Analysis was conducted with STATA 13.1 statistical software (StataCorp, College Station, TX, US) and with R Statistical Software (Foundation for Statistical Computing, Vienna, Austria), using the rms (Frank E Harrell Jr (2018). rms: Regression Modeling Strategies. R package version 5.1-2. https://CRAN.R-project.org/package=rms) and ggplot2 (H. Wickham. ggplot2: Elegant Graphics for Data Analysis. Springer-Verlag New York, 2016) packages.

Results

A total of 836 patients were included in the study. Of them, 358 patients (42.8%) had HCV, 248 (29.7%) NASH, 203 (24.3%) ALD, and 27 patients (3.2%) chronic hepatitis B (HBV). Table 1 shows the baseline characteristics of the different cohorts. Most of the patients were male and had a normal liver function equivalent to Child Pugh score of 5. The prevalence of portal hypertension (HVPG >5 mmHg) in the whole cohort was 83%, while the prevalence of CSPH was 59%.

Differences in baseline characteristics were observed in some etiologies. ALD patients had higher LSM values and a higher prevalence of portal hypertension and CSPH compared to other etiologies. NASH patients had, as expected, a higher body mass index (BMI) than other etiologies, were significantly older and presented higher platelet count. The median HVPG in NASH patients was 7 mmHg, lower than in other etiologies, being the prevalence of portal hypertension and CSPH in NASH patients the lowest.

Prevalence of portal hypertension (HVPG >5 mmHg)

As explained, the prevalence of portal hypertension was different across the distinct etiologies of cACLD patients (Table 1 and 2). HCV and ALD patients were not much different, but NASH patients presented a remarkably lower prevalence of portal hypertension that was maintained across different strata of LSM values (Table 2 and suppl. table 1).

Supplementary table 2 compares the clinical characteristics between NASH patients with and without portal hypertension. As seen, NASH patients without portal hypertension had higher BMI compared to

those with portal hypertension. As seen in table 2, stratifying NASH patients according to BMI showed a much lower prevalence of portal hypertension in all subgroups of obese (BMI ≥30 kg/m^2) patients. In addition, this low prevalence of portal hypertension in obese NASH patients was not due to a lack of use of XL probe for obese patients, since when the analysis was restricted to the NASH patient cohorts with availability of XL probe, portal hypertension prevalence was even lower (Table 2).

Detecting CSPH (HVPG ≥10 mmHg)

Prevalence of CSPH (59%) was different between etiologies of cACLD (Table 1 and figure 1); ALD had the highest prevalence (83.3%) and NASH patients the lowest (39.1%). However, in NASH patients, the prevalence of CSPH changed according to BMI, decreasing the prevalence as BMI increased. The prevalence of CSPH in NASH patients with BMI <30 kg/m^2 was 50.5% (51/101) and 30.8% (41/133) in patients with BMI ≥30 kg/m^2 (Figure 1).

Validation of ANTICIPATE models

The performance of two previously described models from the ANTICIPATE study for risk prediction of CSPH, one using LSM and one using LSM plus platelet count was tested for different etiologies. Those patients already included in the ANTICIPATE study were excluded for this analysis. ALD and HCV patients (etiologies reflecting the composition of the sample in the original ANTICIPATE cohort) were analyzed together, since results were not different when assessed individually. Both models predicted CSPH, demonstrating a very good calibration in the subsample of patients with ALD/HCV etiology (Supplementary figures 1A and 1B). In supplementary figure 2, we illustrate the pointwise predicted risk of individual LSM values by the ANTICIPATE model and the observed accumulated prevalence of CSPH in different ranges of LSM and above a defined value of LSM in ALD plus HCV patients. As shown in the figure, predicted and observed CSPH prevalences increase with increasing LSM values.

In contrast to patients with ALD and HCV, in patients with NASH cirrhosis both models were substantially miscalibrated, considerably overpredicting the risk of CSPH (supplementary figures 1C and 1D).

LSM cut-off for ruling in CSPH

The whole cohort of patients in the study was used to explore the best LSM cut-off for ruling in CSPH with a PPV ≥90%. The accumulated prevalences of CSPH were 83.5% for LSM ≥20 kPa, 91% for LSM ≥25 kPa and 93.7% for LSM ≥30 kPa. Therefore, LSM ≥25 kPa was chosen as the optimal cut-off for ruling in CSPH in our cohort.

When assessing different etiologies, this cut-off performed well for most of the etiologies. There were 144/155 ALD patients (96.6%) with LSM ≥25 kPa and CSPH, 120/130 HCV patients (92.3%) and 11/11 (100%) HBV patients (Figure 1). However, in keeping with the data shown in supplementary figure 1, in NASH patients this cut-off did not provide the pre-specified 90% PPV target; only 57 out of 74 NASH patients (77%) had CSPH above this LSM cut-off. Additional information regarding the performance of the LSM ≥25 kPa for ruling in CSPH is shown in supplementary table 3.

CSPH prediction in NASH patients

Supplementary table 4 shows the differences between NASH patients with LSM ≥25 kPa and CSPH compared to patients without CSPH. Again, one important difference between those groups was BMI. The prevalence of CSPH was higher in non-obese patients with 33/36 patients (91.7%) with LSM ≥25 kPa showing CSPH, while obese patients had lower prevalence of CSPH despite high LSM values (22/35, 62.8%) (Figure 1). Figure 2 represents the predicted probability of CSPH by logistic regression model according to the presence of obesity (BMI ≥30 kg/m^2 vs BMI <30 kg/m^2) (Figure 2A), and according to different BMI values (Figure 2B). For obese NASH patients, the figure shows that the prevalence of CSPH was always <90% whatever the LSM chosen. By contrast, in etiologies other than NASH, BMI did not seem to impact in the effect of LSM to predict the risk of CSPH (Supplementary figure 3).

Since predictions of the ANTICIPATE model were suboptimal in this series and BMI impacted non-invasive predictions of CSPH, we developed a modified nomogram to predict CSPH on the basis of LSM, BMI and platelet count (Figure 3 and supplementary data). Both discrimination (c-statistic 0.90) and calibration (graphically shown in supplementary data) of this refined model were excellent in the present sample.

Association between LSM, Platelet count and HVPG according to BMI in NASH patients

Figure 4 shows the influence of BMI on the non-invasive prediction of HVPG either with LSM alone (Figure 4A) or LSM+platelet count (Figure 4B) in NASH patients. For a given LSM value or a combination of LSM+platelet count values, HVPG was lower for higher BMIs. This trend is also shown (with further granularity) in supplementary figure 4.

<u>Ruling out CSPH: High NPV ≥90%</u>

In the ANTICIPATE model it was not possible to find a population with a very low risk (<10%) of having CSPH. In this cohort, with LSM alone it was also not possible to identify a cut-off with high NPV to exclude CSPH, although in NASH patients the LSM <13.6 kPa had the highest NPV (Table 3). However, adding platelet count ≥150x10^9/L to a LSM ≤15 kPa cut-off could reasonably exclude CSPH in most etiologies

(except for HBV that included a very low number of patients). Additional information regarding the performance of the proposed parameters for ruling out CSPH is shown in supplementary table 3.

Discussion

The present study demonstrates that LSM alone is useful to select patients with chronic liver disease at risk of having portal hypertension and CSPH for most etiologies, including ALD, HCV and HBV. At the same time, our work illustrates that in NASH patients, and especially obese NASH patients, these LSM-based rules behave differently from other etiologies, in part because the association between LSM and HVPG changes with BMI in patients with NASH. It is important to keep in mind that our aim was to analyze the relationship between LSM and HVPG values in populations that were selected based on LSM values and not on histological staging or clinical features.

The first important hallmark of our study was the differences in portal hypertension prevalence among patients with cACLD from different etiologies, selected on the basis of a LSM 10 kPa. Patients with HCV, ALD and HBV presented prevalences above 90% that increased to nearly 100% when subgroups with higher LSM values were selected. By contrast, NASH patients had a lower portal hypertension prevalence in the whole population and also in the different subgroups determined by higher LSM values. Obesity seemed to be, in part, responsible for the lower prevalence of portal hypertension, since obese patients (BMI ≥30 kg/m^2) showed even lower rates of portal hypertension as compared to other etiologies and nonobese NASH patients. Recent studies have suggested that obesity can reduce the diagnostic accuracy of LSM for advanced liver fibrosis in NASH patients by increasing the rates of false positives; this worse diagnostic performance is also seen when M or XL probes are used [20]. In addition, Wong, et al [21] have shown that obesity might be responsible for higher LSM values as compared to nonobese NASH patients with the same fibrosis stage. A similar situation might be taking place in our study, generating lower rates of portal hypertension in NASH patients as compared to other etiologies within the same LSM values strata. Also, in our study, the availability of the XL probe for the prospective cohort of NASH patients did not resulted in an increased prevalence of portal hypertension, but rather the opposite.

Another objective of our study was to develop classification rules for diagnosing and excluding CSPH in the different etiologies of cACLD patients that could be readily used in clinical practice. Baveno VI recommendations were restricted to viral etiologies and the proposed rules were not clearly defined [1]. Our data indicate that LSM ≥25 kPa is sufficient to rule in CSPH in ALD, HBV, HCV and nonobese NASH patients with PPV higher than 90%, without the need of additional noninvasive parameters. In terms of applicability, having a single parameter with a single cut-off will undoubtedly facilitate the point-of-care

use of the classification rule by clinicians. Considering that prophylactic measures to prevent cirrhosis decompensation, such as β-blockers, might be established as standard of care in patients with CSPH in the future [6], the simple rules presented here would clearly help in patient selection. In addition, the ANTICIPATE model for predicting CSPH also showed a very good calibration in the population of ALD and HCV patients, indicating that it is a robust model when applied to populations with etiologies similar to the sample where it was derived. This model could also be utilized to select patients at risk of CSPH, especially patients with LSM between 15 and 25 kPa.

Again, a very different scenario was found in NASH patients, especially obese NASH patients. Taken together, the information regarding NASH patients can be summarized as follows: 1) the prevalence of CSPH in the NASH population with LSM ≥25 kPa is lower (77%) than in other etiologies resulting in an insufficient PPV; 2) obese NASH patients (BMI ≥30 kg/m^2) with LSM ≥25 kPa are the subgroup with the lowest prevalence of CSPH (62.8%); 3) the ANTICIPATE model overpredicts the presence of CSPH in these patients (Supplementary figure 1C and D); 4) obesity seems to influence LSM prediction of CSPH only in NASH patients (Figure 2A and B), as opposed to obese non-NASH patients (supplementary figure 3); 5) the impact of BMI on LSM predictions of CSPH increases with higher BMIs (Figure 2B); and 6) Increasing LSM cut-off did not improve sufficiently CSPH prediction in NASH patients. As an alternative, a new model for NASH patients (ANTICIPATE-NASH model) to predict CSPH was developed using LSM, platelet count and BMI, and a nomogram for practical use constructed.

One issue that is relevant for interpreting the data presented here refers to the basal differences between the distinct etiologic groups of patients, and especially the NASH group from the others. In this study, using the same inclusion criteria in all patients, NASH patients were different in terms of prevalence of portal hypertension and CSPH, having the lowest prevalence of the total cohort. It could be argued that this might have affected the results in terms of prediction; the lowest the prevalence of a feature, the lowest the probability of having high PPV. The different cohorts evaluated here are a reflection of the type of patients currently seen in tertiary hospitals. For the case of NASH patients, many of them are probably referred from primary care centers for suspicion of advanced disease and evaluated for entering NASH clinical trials, selecting a less sick population and consequently introducing a selection bias in the analysis. However, it is not less true that all patients were included based on an objective cut-off LSM value and the percentage of LSM <20 kPa in NASH patients was not different from HCV and HVB patients. Also, as explained before, the differences in prevalence of portal hypertension and CSPH observed in NASH patients were preserved in all sub-strata of LSM values, excluding a selection bias of the recruited population as the main cause for this difference. Thus, the issue is why NASH patients with the same LSM values (or range of LSM values) showed less portal hypertension and CSPH than other etiologies or in

other words, what lies behind the observation that obese NASH patients have less severe liver disease, despite similar LSM values. This cannot be answered in the present study and many other reports have given contradictory results regarding the effect of body and liver fat in LSM readings [20,22–25]. In a study by Petta, et al. performed in 253 patients with NASH and using only M probe, they found that in patients with low fibrosis (F0 to F2) median values of LSM were higher in those patients with severe steatosis at liver biopsy compared to those without, increasing the rates of false positive LSM values [23]. Similar data was obtained later on in a study with 2058 patients with mixed etiologies (18% NASH) where they found a slight effect of steatosis in increasing LSM values in patients with absent or mild fibrosis, but not in more advanced stages of fibrosis [24]. However, recently, a multicentric study including 450 NASH patients with paired liver biopsy and LSM (using M or XL probes as needed) found that the only parameter affecting LSM values was fibrosis stage and no association was found with steatosis or probe type [22]. None of these previous studies evaluated BMI as a possible factor that could influence LSM values. Wong, et al. found that obese NASH patients (BMI ≥30 kg/m^2) had higher LSM than those with BMI <30 kg/m^2 at all fibrosis stages in liver biopsy and for both M and XL probes [21]. Myers, et al. evaluated factors influencing discordance in fibrosis staging using XL probe and they found that the only factor associated with fibrosis staging discordance between liver biopsy and LSM was BMI, overestimating liver fibrosis measured by LSM [25]; patients with BMI ≥40 kg/m^2 and LSM ≥7 kPa had the highest rates of discordance. Therefore, it remains unclear whether LSM inaccuracy in NASH patients is just a "technical issue" in obese people related to skin-to-liver distance or other physical characteristics, or if fat might increase liver stiffness without fibrosis. Liver biopsy information from our patients, unfortunately unavailable, might have shed some light in clarifying these possible mechanisms. What it is very clear is that overall in NASH patients, LSM is not a good predictor of advanced fibrosis and cirrhosis with very low PPV and many different cut-offs proposed [23,26]; this inconsistency is probably reflected here when predicting CSPH in these patients.

Finally, in terms of excluding CSPH in our cohort with a NPV of ≥90%, we observed that LSM ≤15 kPa plus platelet count ≥150x10^9/L was useful for ruling out CSPH in the whole cohort and in all etiologies. However, in HBV, the number of patients was too low to confirm the rule.

Our study has some limitations. The two main cohorts were retrospective and mainly composed of HCV and ALD patients, lacking the XL probe in all LSM performed. The newer ones enriched with NASH patients were not specifically recruited for the present study and not all NASH patients could have the possibility of using the XL probe. Using LSM by transient elastography as the main non-invasive parameter is a limitation for countries or centers with no availability for the technique. Also, concomitant treatments that might have influenced HVPG values (β-blockers, statins and others) have not been considered in our

study. On the other hand, differences in prevalences of portal hypertension or CSPH could have influenced the results of PPV or NPV. However, as mentioned before, this cohort is a reflection of the patients we currently see in our tertiary hospitals. We also acknowledge that selecting patients, mainly NASH patients, with a LSM ≥10 kPa might have prevented detecting patients with portal hypertension and no or mild fibrosis, as it has been reported in a few studies (27,28). Finally, we acknowledge the need of future validation of the NASH patient data, including the lowest prevalences of portal hypertension and CSPH, and the utility of the proposed ANTICIPATE-NASH patient model.

The strengths of the present study are that this is a large multicentric cohort including a large number of patients with the main etiologies of chronic liver disease (especially the new data on NASH patients), increasing the external validity of its results. Our classification rules are simple rules that can be applied in daily clinical practice without the need of complex calculations. Figure 5 summarizes the different LSM cut-offs indicative of cACLD and CSPH.

In conclusion, the present study shows that in patients with cACLD (defined as a LSM >10 kPa) portal hypertension is present in more than 90% when the etiology is ALD, HCV and HBV, whereas in NASH patients with cACLD, and especially in those with obesity, prevalence of portal hypertension is much lower. Regarding the non-invasive estimation of CSPH, Baveno VI LSM ≥25 kPa cut-off is sufficient to rule in CSPH in ALD, HBV, HCV and nonobese NASH patients, but is not specific enough in obese NASH patients. Finally, LSM ≤15 kPa plus platelet count ≥150x10^9/L can rule out CSPH in most etiologies of chronic liver disease.

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Figure legends

Figure 1. Prevalence of clinically significant portal hypertension (CSPH) by etiologies including all patients in each group (in blue) and only patients with liver stiffness measurement (LSM) ≥25 kPa (in red). Non obese NASH: BMI <30 kg/m^2; obese NASH: BMI ≥30 kg/m^2.

Figure 2. Predicted probability by a logistic regression model of CSPH in NASH patients according to: A) the presence of obesity (body mass index (BMI) ≥30 kg/m^2 in red color line) or not (BMI <30 kg/m^2 in blue color line); B) different BMI.

Figure 3. Nomogram to predict the presence of clinically significant portal hypertension (CSPH) in patients with non-alcoholic steatohepatitis (NASH) using the variables liver stiffness measurement (LSM), body mass index (BMI) and platelet count. ~~The number of points per each variable are estimated with the first line (Points), then the summation of points of the three variables will indicate the probability of CSPH by applying the total points line to the CSPH line.~~ To obtain the risk of CSPH trace a vertical line from each of the three predictors' axis to the first line ("points"). Add the total points and trace a vertical line from the "total points" axis to the probability axis to calculate the risk of CSPH. As shown, a patient with a LSM value of 20 kPa (29 points), a BMI of 35 (9 points) and a platelet count of 150x10^9 (67 points) would have a predictive probability of CSPH of 40% (for a total of 105 points).

Figure 4. Influence of body mass index (BMI) on the predicted values of hepatic venous pressure gradient (HVPG), based on liver stiffness measurement (LSM) alone or with LSM+platelets in NASH patients. As shown in panel A, for any given value of LSM, mean HVPG values are lower for higher BMIs. Panel B shows a similar observation. For any given combination of LSM and platelet count, predicted mean HVPG is lower as BMI increases. These figures are the graphical representation of the linear regression models outlined in the supplementary data.

Figure 5. The "rule of five". Proposed liver stiffness measurement cut-offs for ruling in and out compensated advanced chronic liver disease (cACLD) and clinically significant portal hypertension (CSPH), in addition to the Baveno criteria for avoiding screening endoscopy. Information was extracted from data from this study and the Baveno VI recommendations [1]. Plat: platelet count; HCV: Chronic hepatitis C; HBV: Chronic hepatitis B; ALD: Alcoholic liver disease; NASH: Non-alcoholic steatohepatitis

Figure

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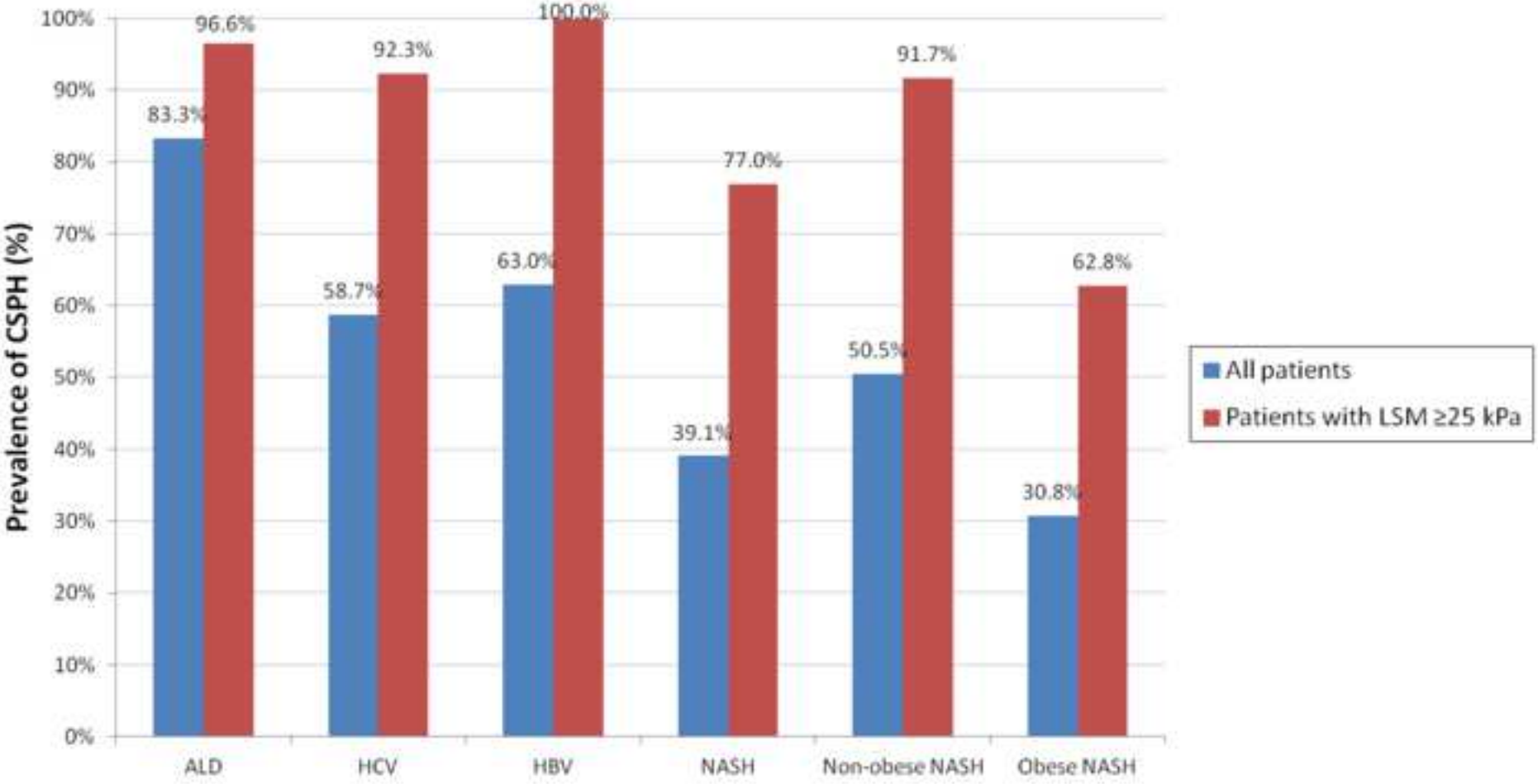

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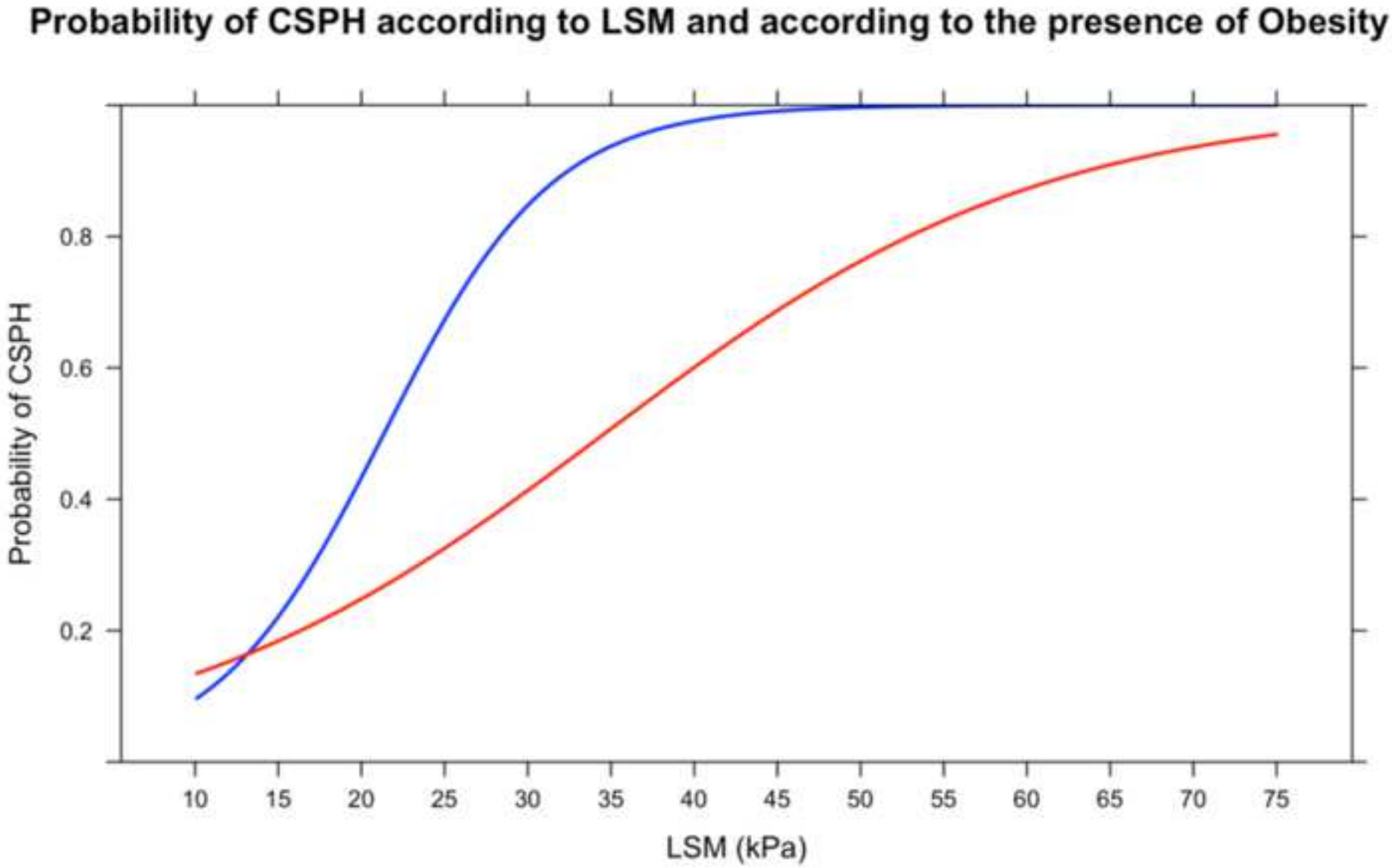

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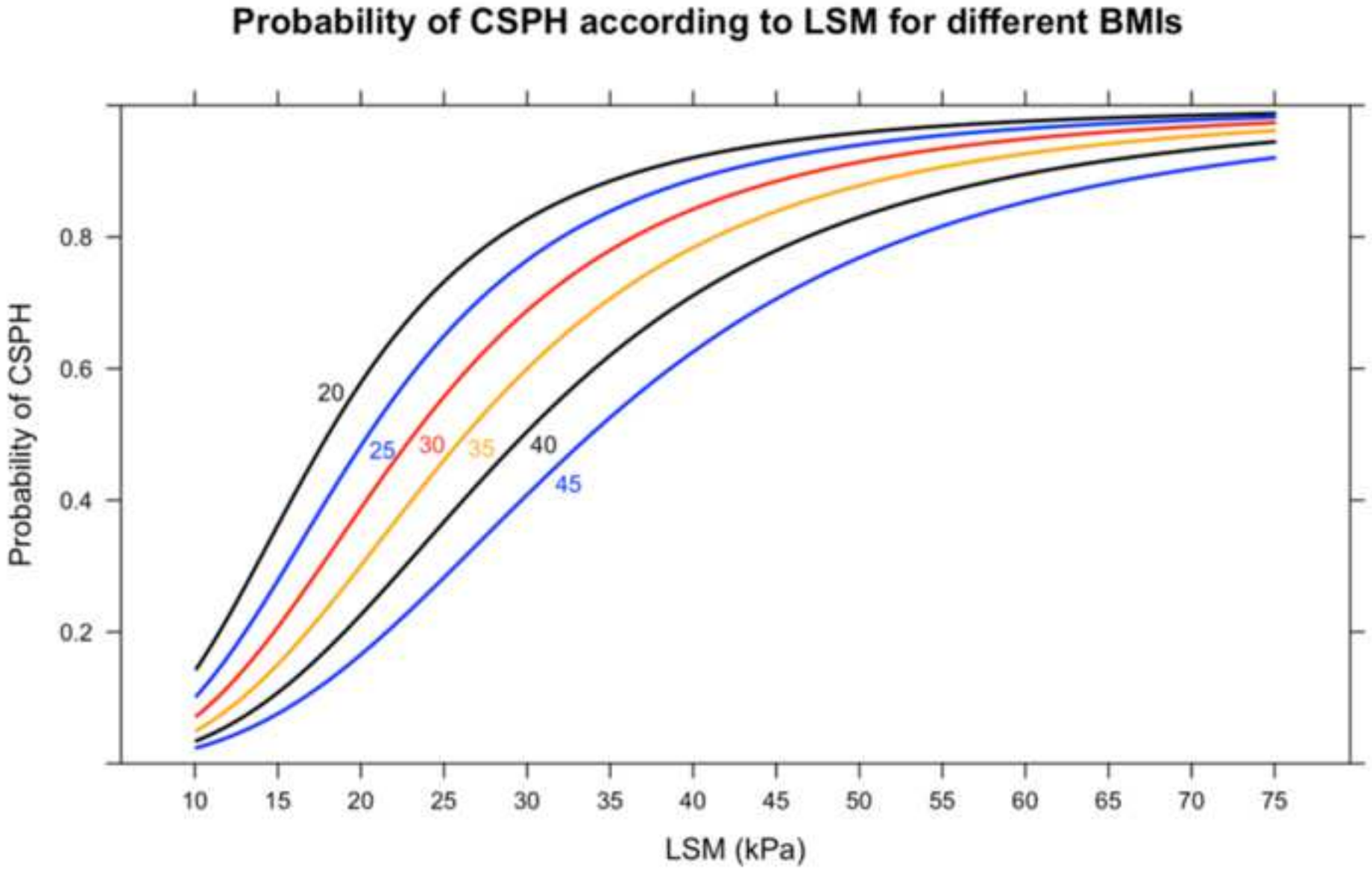

Figure

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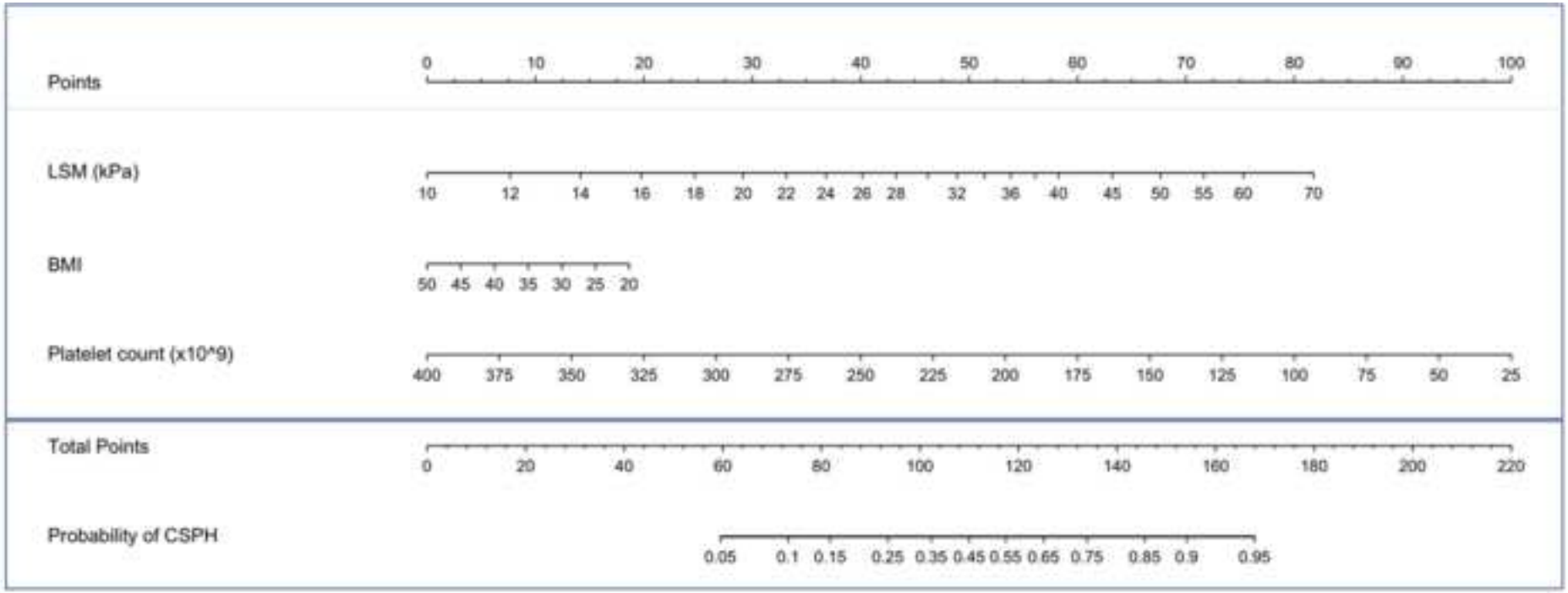

Figure

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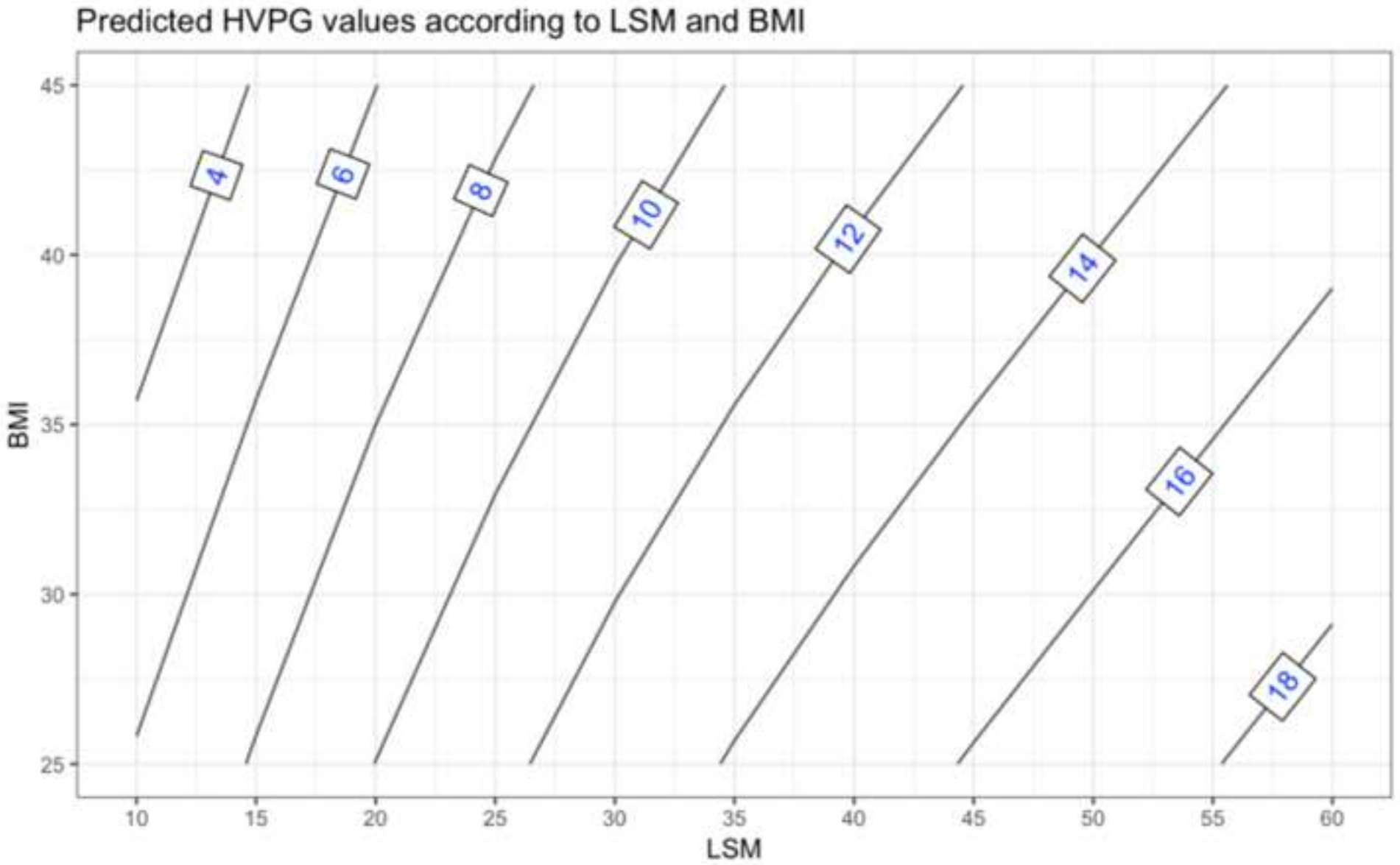
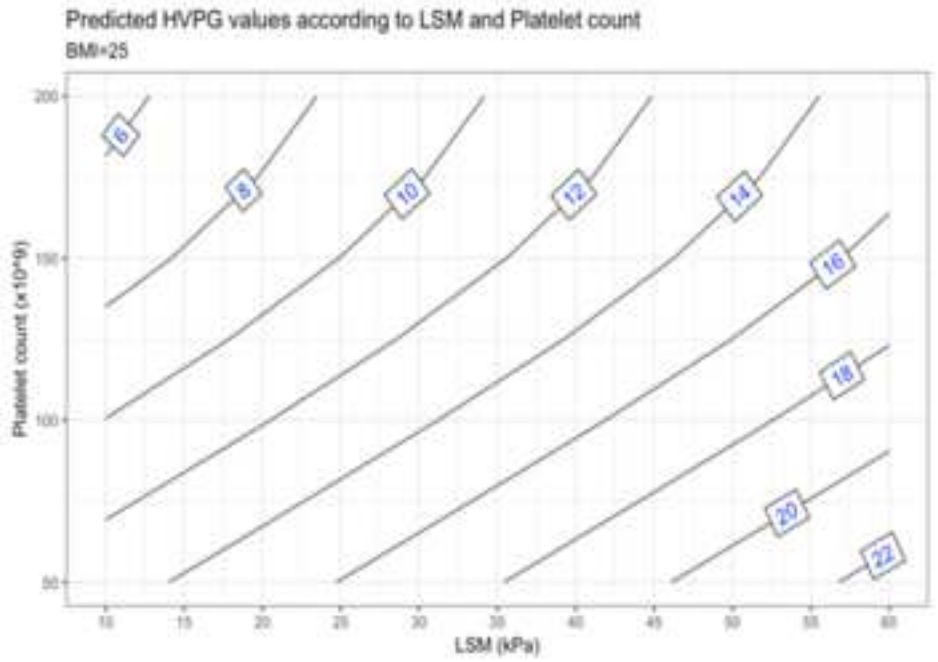
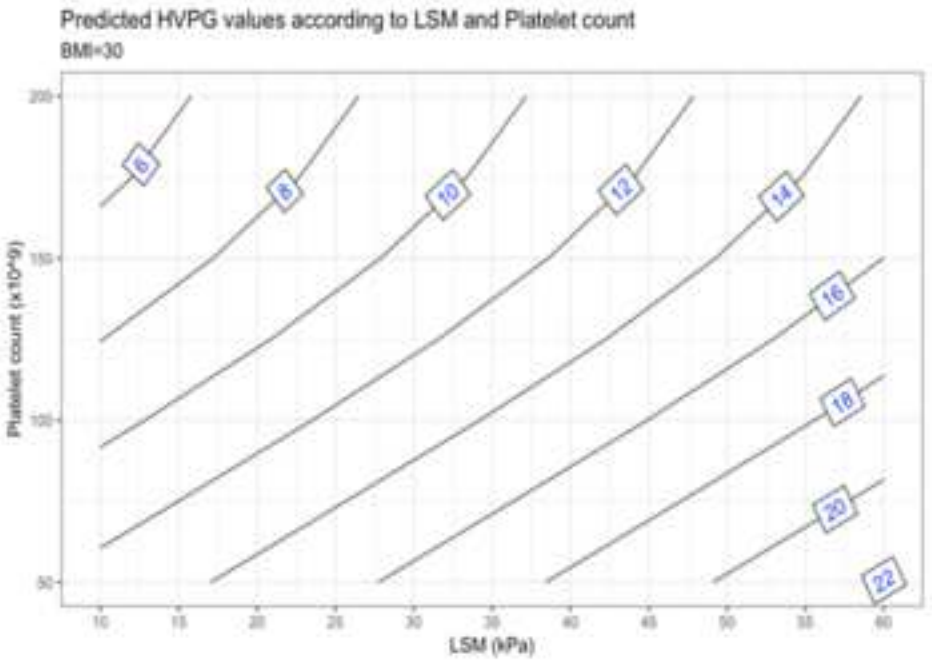
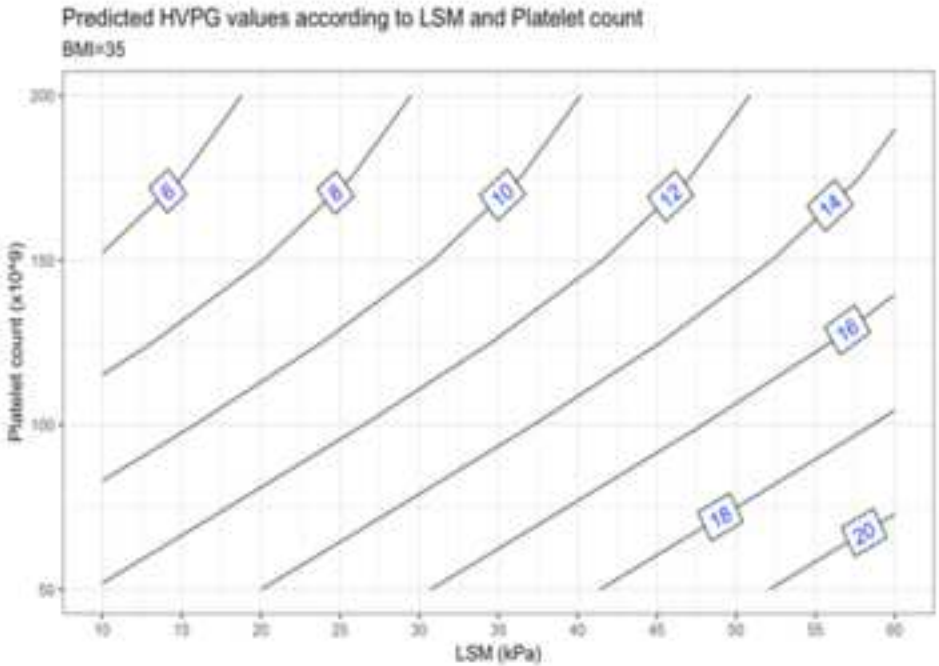


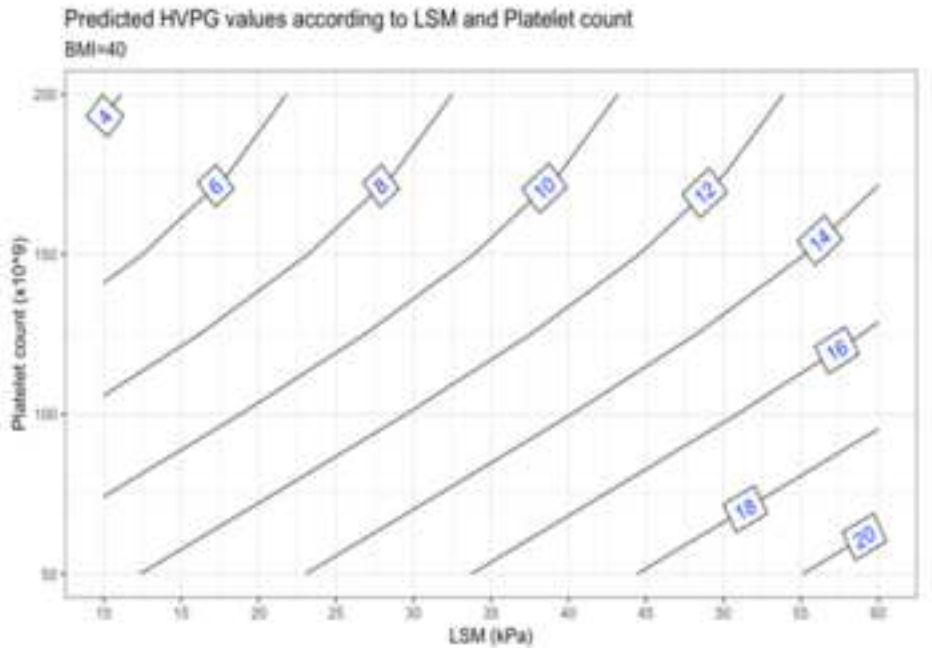
Figure

Click here to access/download;Figure;Figure4B.tif









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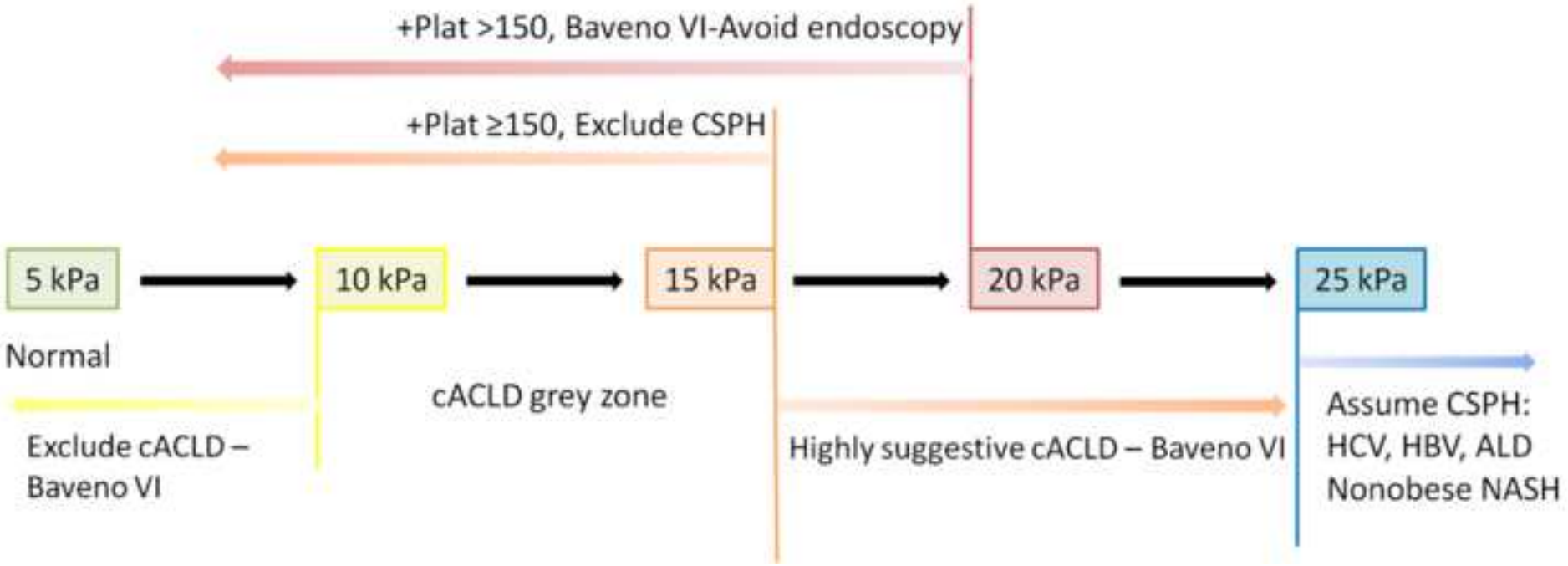

TABLES

Table 1. Baseline characteristics of patients based on etiologies.

Characteristics	All N=836	ALD N=203	HCV N=358	NASH N=248	HBV N=27	P value
Age, years	57 (49-66)	58 (49-65)	55 (48-64) *	61 (54-68.5) *,§	52 (45-60) §	<0.001
Male, n (%)	534 (63.8)	135 (66.5)	235 (65.6)	148 (59.6)	16 (59.2)	0.357
BMI, kg/m^2	26.8 (23.7-31)	25.6 (22.3-28.4)¥	25.4 (23.1-28.3)*	30.9 (27.3-36) *,§,¥	24.6 (19.8-27.7) §	<0.001
Platelets, x10^3/µL	128 (88.5-179.5)	123.5 (87-166)¥	115 (78-153) *	166.5 (113.5-226) *,¥	138.5 (98-171)	<0.001
Albumin, g/dL	3.97 (3.66-4.21)	3.89 (3.54-4.12)¥,¤	3.98 (3.69-4.24)¤	4.03 (3.7-4.3)¥	4 (3.7-4.3)	0.001
Bilirubin, mg/dL	1 (1-1.2)	1.09 (1-1.44)¥,¤	1 (1-1.15)¤	1 (1-1.03)¥	1 (1-1.23)	<0.001
Creatinine, mg/dL	1 (1-1)	1 (1-1)	1 (1-1)	1 (1-1.01)	1 (1-1)	0.083
INR	1.1 (1-1.2)	1.2 (1.1-1.3) ¥,¤	1.1 (1-1.2) ¤,*	1.1 (1-1.1) *,§,¥	1.2 (1-1.3) §	<0.001
AST, U/L	55 (37-86)	47 (34-69) ¤	76 (47-108) ¤,*	45 (33-64) *	60 (42-88)	<0.001
ALT, U/L	52 (32-91)	35 (24-51) ¤	78 (44-119) ¤,*	47.5 (31-69) *	49 (38-66)	<0.001
GGT, U/L	122 (64-218)	138 (75-235)	106.5 (59-182) *	125 (64-259) *	84 (46.5-144)	0.011
FIB4	3.6 (2.1-5.7)	3.9 (2.4-6.4)¥	4.3 (2.6-6.6) *	2.6 (1.4-4.3) *,¥	3.2 (2.3-5.1)	<0.001
Child-Pugh, n (%) **5 points** **6 points**	 652 (78) 184 (22)	 146 (72.3) 57 (27.7)	 283 (79) 75 (21)	 201 (81.1) 47 (19)	 22 (81.5) 5 (18.5)	0.425
MELD	8 (7-10)	9 (8-11) ¥,¤	8 (7-10) ¤	7 (6-9) ¥	8 (8-10)	<0.001
LSM, kPa	21.8 (15-34.3)	35.3 (22.8-63.9)¥,¤,±	20.6 (15-29.1) ¤	18.4 (13.1-26.8) ¥	20 (13.9-32.8) ±	<0.001
LSM <20 kPa, n (%)	351 (42)	35 (17.2) ¥,¤,±	170 (47.5) ¤	133 (53.6) ¥	13 (48.2) ±	<0.001
HVPG, mmHg	11 (7-17)	15 (11-20) ¤,¥	11 (8-16) ¤,*	7 (4-13)¥,*,§	12 (7-18)§	<0.001
PH, n (%)	694 (83)	196 (96.6)¥	322 (89.9)*	151 (60.9) ¥,*,§	25 (92.6)§	<0.001
CSPH, n (%)	493 (59)	169 (83.3) ¤,¥	210 (58.7) ¤,*	97 (39.1) ¥,*	17 (63)	<0.001

Continuous variables represented as median (percentile 25- percentile 75). The Child-Pugh and MELD scores were used to illustrate liver function acknowledging that some patients do not have cirrhosis.

ALD: Alcoholic liver disease; HCV: Chronic hepatitis C; NASH: Non-alcoholic steatohepatitis; HBV: Chronic hepatitis B; BMI: Body mass index; AST: Aspartate aminotransferase; ALT: Alanine-aminotransferase; GGT: Gamma-glutamyl transferase; FIB4: Fibrosis-4 score; MELD: Model for end-stage liver disease; LSM: Liver stiffness measurement; HVPG: Hepatic venous pressure gradient; PH: Portal hypertension; CSPH: Clinically significant portal hypertension.

Paired comparisons corrected by the Bonferroni method: P<0.05 between the following groups: * HCV and NASH/ § HBV and NASH/ ¥ ALD and NASH/ ¤ ALD and HCV/ ± ALD and HBV.

Table 2. Prevalence of portal hypertension (HVPG >5 mmHg) among all etiologies of patients with compensated advanced chronic disease in the study and according to different strata of liver stiffness values (LSM). HVPG: Hepatic venous pressure gradient; ALD: Alcoholic liver disease; HCV: Chronic hepatitis C; NASH: Non-alcoholic steatohepatitis; HBV: Chronic hepatitis B.

Patients	**LSM ≥10 kPa (all patients within the group)**	**LSM >15 kPa**	**LSM >20 kPa**	**LSM >25 kPa**
All patients	694/836 (83%)	564/625 (90.2%)	448/475 (94.3%)	354/365 (97%)
ALD	196/203 (96.6%)	176/179 (98.3%)	162/164 (98.7%)	149/150 (99.3%)
HCV	322/358 (90%)	253/268 (94.4%)	179/185 (96.7%)	129/130 (99.2%)
All NASH	151/248 (60.9%)	116/159 (73%)	94/113 (83.2%)	65/74 (87.8%)
Non-obese NASH*	69/101 (68.3%)	55/66 (83.3%)	46/52 (88.5%)	35/37 (94.5%)
Obese NASH*	71/133 (53.3%)	54/85 (63.5%)	43/56 (76.8%)	28/35 (80%)
All NASH-XL§	86/170 (50.5%)	61/97 (62.9%)	47/63 (74.6%)	29/38 (76.3%)
Non-obese NASH-XL§	28/50 (56%)	21/28 (75%)	18/23 (78.2%)	11/13 (84.6%)
Obese NASH-XL§	53/112 (47.3%)	37/66 (56.1%)	30/40 (75%)	18/25 (72%)
HBV	25/27 (92.6%)	19/19 (100%)	--	--

* NASH patients stratified according to body mass index (<30 kg/m^2: non-obese; ≥30 kg/m^2: obese).

§ NASH-XL: refers to patients with NASH evaluated with Fibroscan 502 Touch in which M and XL probe was available.

Table 3. Negative predictive value of different LSM cut-offs and also adding platelet count to rule out the presence of CSPH in different etiologies.

LSM cut-off	Etiology	N Patients*	HVPG <10 mmHg§	NPV (95% CI)
<15 kPa	ALD	23	17	73.9 (53.5-87.5)
	HCV	87	71	81.6 (72.2-88.4)
	NASH	85	75	88.2 (79.7-93.5)
	HBV	8	5	62.5 (30.6-86.3)
	All	**203**	**168**	**82.8 (77-87.3)**
<13.6 kPa	ALD	16	11	68.8 (44.4-85.8)
	HCV	63	54	85.7 (75-92.3)
	NASH	64	57	89.1 (79.1-94.6)
	HBV	5	4	80 (37.6-96.4)
	All	**148**	**126**	**85.1 (78.5-90)**
≤15 kPa + Platelets ≥150x10^9/L	ALD	12	12	100 (75.8-100)
	HCV	34	34	100 (89.8-100)
	NASH	66	63	95.5 (87.5-98.4)
	HBV	5	4	80 (37.6-96.4)
	All	**117**	**113**	**96.6 (91.5-98.7)**

LSM: Liver stiffness measurement; HVPG: Hepatic venous pressure gradient; NPV: Negative predictive value; CI: Confidence interval; ALD: Alcoholic liver disease; HCV: Chronic hepatitis C; NASH: Nonalcoholic steatohepatitis; HBV: Chronic hepatitis B.

* Number of patients within LSM cut-off.

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* Number of patients within LSM cut-off.

§ Number of patients without clinically significant portal hypertension within the LSM cut-off.

SUPPLEMENTARY DATA

Study cohorts

Patients excluded from the different cohorts:

-ANTICIPATE cohort: the ANTICIPATE study had originally 229 patients with HVPG and LSM pairs. A total of 13 patients were excluded: 3 without etiology, one with primary biliary cholangitis and 9 with rare etiologies.

-Vienna retrospective cohort: of the 1908 patients belonging to different studies, 1508 were excluded. The reasons for exclusion were: 550 duplicates, 418 LSM <10 kPa, 262 unreliable LSM (IQR/M >30%), 100 Child-Pugh class B/C, 72 prior decompensation, 49 varices on β-blocker therapy, 33 rare etiologies, 15 more than 3 months between LSM and HVPG, and 9 cholestatic liver diseases.

-Toulouse cohort: of the 100 patients included in the original cohort, 8 were excluded because of Child-Pugh class B and 18 because of unreliable LSM.

-Vienna cohort: the second Vienna cohort had 73 patients, from that 23 patients were excluded because of unreliable LSM (9), Child-Pugh class B/C (5), rare etiologies (3) and cholestatic/autoimmune diseases (6).

-Bern cohort: the original cohort had 40 patients, and 2 patients with autoimmune hepatitis and one with primary biliary cholangitis were excluded.

-London cohort: there were 57 patients in the cohort and 12 were excluded because of LSM <10 kPa, 6 with Child-Pugh class B/C, 2 rare etiologies, one autoimmune hepatitis, one primary sclerosing cholangitis, and one with unreliable LSM.

-Barcelona cohort: no exclusions.

Statistics

a) Association between body mass index (BMI), liver stiffness measurement (LSM) by transient elastography (TE) + platelet count with the presence of clinically significant portal hypertension (CSPH):

The association between Platelet count, LSM and BMI with the probability of CSPH was modelled with logistic regression. The full model was better than any reduced model according to Akaike information criterion. No non-linear terms added to the predictive capacity of the model. LSM was log transformed. Model was corrected for optimism with bootstrap.

Final model:

```
logit = -3.9529402 + 2.2835809 * log(LSM) - 0.033777725 * BMI -
0.014490895 * Plat

LSM: liver stifness measurement in kPa

Plat: platelet count (x10^9)
```

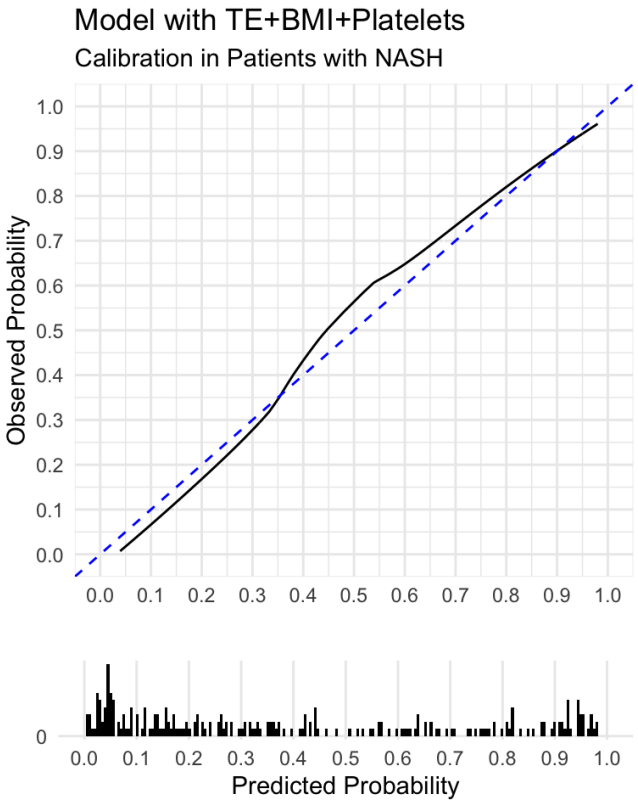
Bootstrap-corrected performance indices:

C-statistic= 0.90

R2=0.57

Individual probabilities of CSPH can be calculated as 1/(1+exp(-logit))

Calibration plot of the bootstrap corrected model:



The histogram below the figure shows the distribution of patients according to their calculated risks in the current samples.

Figure 3 of the main manuscript represents the nomogram derived from this model.

b) Influence of body mass index (BMI) on the association between liver stiffness measurement (LSM) by transient elastography (± platelet count) and hepatic venous pressure gradient (HVPG):

For assessing the influence of BMI on the association between TE and HVPG, we conducted a multiple linear regression in which HVPG was the dependent variable and LSM and BMI the independent variables. Non-linear terms were explored with restricted cubic splines. Final models were selected on the basis of Akaike information criterion.

R2 of the model	0.433
Significance testing	
	P
Liver Stiffness (kPa)(total significance)	<.0001
Nonlinear term for LSM	0.038
BMI	<.0001

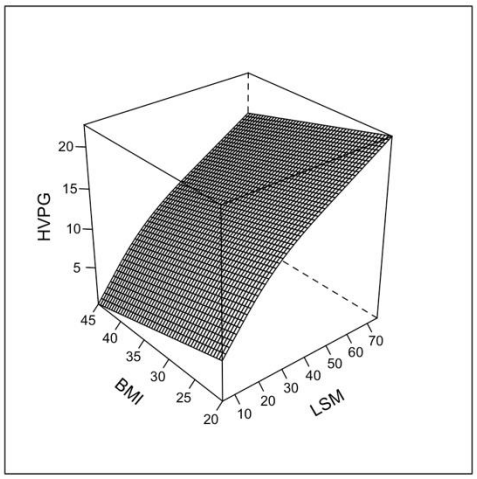
Model Equation

```
HVPG =  7.1758437 + 0.40441814 * LSM - 0.00026646376 * pmax(LSM -
        11.07, 0)^3 + 0.00032847962 * pmax(LSM - 18.35, 0)^3 -
        6.2015862e-05 * pmax(LSM - 49.63, 0)^3 - 0.20221371 * BMI


LSM: liver stifness measurement in kPa

Note: pmax is a function of R base that returns the maximum value of
the two terms separated by the comma. For example, pmax(3,0) would
return a 3, whereas pmax(-1,0) would return a 0.
```

Graphical representation (Figure 4A in the main manuscript shows a different graphical representation of this model).



We used a similar approach for assessing the influence of BMI on the association between LSM+Platelet count and HVPG. In this case, we conducted a multiple linear regression in which HVPG was the dependent variable and TE, Platelet count and BMI were the independent variables. Non-linear terms were explored with restricted cubic splines.

R2 of the model 0.561

Significance testing

	P
Liver Stiffness (kPa)	<.0001
BMI	0.0113
Plat (x109)	<.0001
Nonlinear term for Plat	0.0001

Model Equation

```
HVPG = 17.415133 + 0.18713102 * LSM - 0.11251463 * BMI - 0.064557963 *
        Plat + 1.0335425e-06 * pmax(Plat - 75.7, 0)^3 - 1.8281712e-06
        * pmax(Plat - 166.5, 0)^3 + 7.9462877e-07 * pmax(Plat -
        284.6, 0)^3


LSM: liver stiffness measurement in kPa

Plat: platelet count (x10⁹)

Note: pmax is a function of R base that returns the maximum value of
the two terms separated by the comma. For example, pmax(3,0) would
return a 3, whereas pmax(-1,0) would return a 0.
```

Figure 4B in the main manuscript shows a graphical representation of this model.

Supplementary table 1. Prevalence of portal hypertension (HVPG >5 mmHg) in different etiologies of compensated advanced chronic liver disease according to different strata of liver stiffness measurement (LSM). HVPG: Hepatic venous pressure gradient; ALD: Alcoholic liver disease; HCV: Chronic hepatitis C; NASH: Non-alcoholic steatohepatitis; HBV: Chronic hepatitis B.

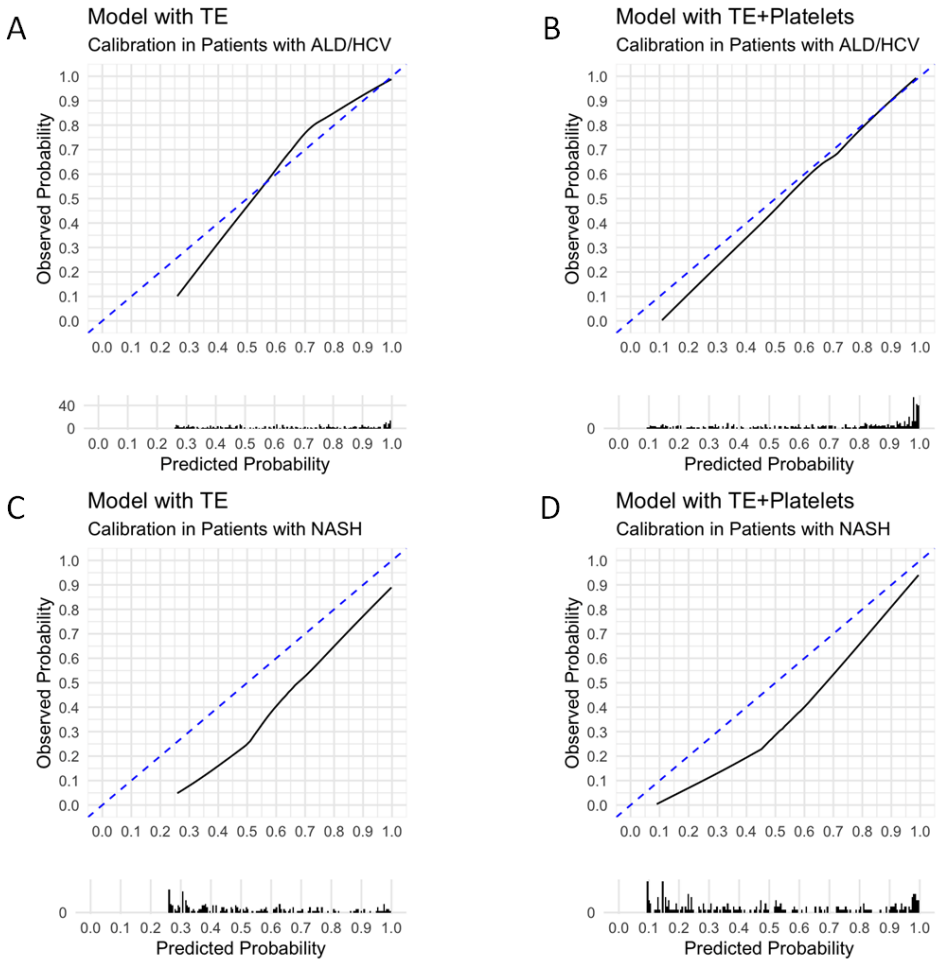
Patients	**LSM 10-15 kPa**	**LSM >15-20 kPa**	**LSM >20-25 kPa**
ALD, n=203	20/24 (83.3%)	14/15 (93.3%)	13/14 (92.8%)
HCV, n=358	69/90 (76.7%)	74/83 (89.1%)	50/55 (90.9%)
NASH, n=248	35/89 (39.3%)	22/46 (47.8%)	29/39 (74.3%)
HBV, n=27	6/8 (75%)	6/6 (100%)	2/2 (100%)
All, n=836	130/211 (61.6%)	116/150 (77.3%)	94/110 (85.4%)

Supplementary table 2. Characteristics of patients with NASH according to the presence of portal hypertension.

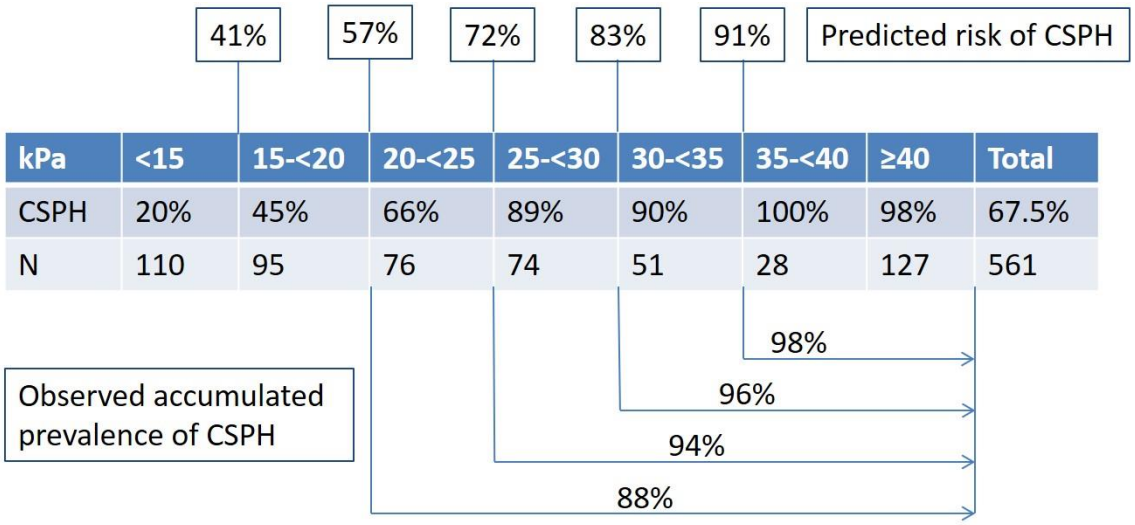
Characteristics	HVPG ≤5 mmHg N= 97	HVPG >5 mmHg N=151	P value
Age, years	59 (50-67)	61 (55-69)	**0.019**
Male, n (%)	61 (62.9%)	87 (57.6%)	0.409
BMI, kg/m^2	33.6 (28.4-38.1)	30.1 (26.3-34.6)	**0.001**
Platelets, x10^9/L	227 (180-271)	125 (93-180)	**<0.001**
Albumin, g/L	41 (37.6-43.7)	40 (37-42.8)	0.065
Creatinine, umol/L	88.4 (88.4-91.1)	88.4 (88.4-89.3)	0.406
AST, IU/L	39 (29-60)	50 (37-65)	0.624
ALT, IU/L	52 (33-74)	44 (30-65)	**0.011**
GGT, IU/L	91 (47-212)	149 (79-283)	0.528
FIB4	1.4 (1-2.1)	3.5 (2.4-5.5)	**<0.001**
Child-Pugh, n (%) **5 points** **6 points**	 88 (90.7) 9 (9.3)	 113 (74.8) 38 (25.2)	**0.002**
MELD	7 (6-8)	8 (7-10)	**<0.001**
LSM, kPa	14.1 (11.8-17.3)	22.8 (16-41)	**<0.001**

HVPG: Hepatic venous pressure gradient; BMI: Body mass index; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; GGT: Gamma-glutamyl transferase; FIB4: Fibrosis-4 score; MELD: Model for end-stage liver disease; LSM: Liver stiffness measurement.

Supplementary figure 1. Performance of the ANTICIPATE models to predict clinically significant portal hypertension (CPSH) in the new sample of patients. The plots show the agreement between predicted and observed rates of CSPH (calibration) with transient elastography (TE) alone (Panels A and C) or with the combination of TE and platelet count. Panels A and B show the performance of the model in patients with alcohol (ALD) or hepatitis C (HCV) related cirrhosis. Panels C and D show the performance in patients with NASH related cirrhosis. Calibration was very good in patients with ALD/HCV, while the models overpredicted the rate of CSPH in patients with NASH. The histograms below each figure show the distribution of patients according to their calculated risks in the current samples.



Supplementary figure 2. Comparison between the predicted risk of ANTICIPATE model (upper percentages in boxes) per single LSM value, as indicated in the kPa line (example, 41% refers to 15 kPa LSM value) in HCV and ALD patients and the accumulated prevalence per ranges of LSM values (middle line of percentages) or all patients above a certain LSM value (lower percentages with arrows).



Supplementary table 3. Diagnostic performance parameters of LSM >25 kPa for ruling in CSPH (A) and LSM ≤15 and platelet count ≥150 for ruling out CSPH (B).

A

	CSPH	No CSPH	Se	Sp	LR+	LR-	Dx Acc	NPV	PPV
ALL (n=836)	332	25	0.67 (0.55-0.62)	0.90 (0.87-0.93)	7.0 (5.0-9.7)	0.36 (0.32-0.41)	0.77 (0.74-0.80)	0.65 (0.61-0.70)	**0.91 (0.87-0.93)**
HCV (n=358)	120	10	0.57 (0.50-0.63)	0.93 (0.87-0.96)	8.46 (4.60-15.56)	0.46 (0.39-0.54)	0.72 (0.67-0.77)	0.60 (0.54-0.67)	**0.92 (0.86-0.96)**
HBV (n=27)	11	0	0.65 (0.39-0.85)	1 (0.66-1)	inf	0.35 (0.19-0.67)	0.78 (0.58-0.91)	0.63 (0.36-0.84)	**1 (0.68-1)**
ALD (n=203)	144	6	0.85 (0.79-0.90)	0.82 (0.65-0.93)	4.82 (2.33-10.0)	0.18 (0.12-0.26)	0.85 (0.79-0.89)	0.53 (0.39-0.66)	**0.96 (0.91-0.98)**
NASH (n=248)	57	17	0.59 (0.48-0.69)	0.89 (0.82-0.93)	5.22 (3.23-8.42)	0.47 (0.37-0.59)	0.77 (0.71-0.82)	0.77 (0.70-0.83)	**0.77 (0.66-0.86)**

CSPH: clinically significant portal hypertension; Se: sensitivity; Sp: specificity; LR+: positive likelihood ratio; LR-: negative likelihood ratio; Dx Acc: overall diagnostic accuracy; NPV: negative predictive value; PPV: positive predictive value

B

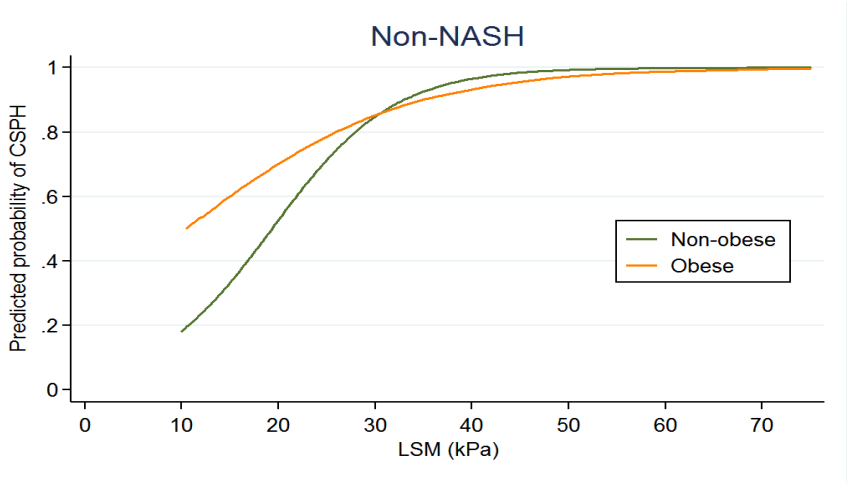
	CSPH	NO CSPH	SE	SP	LR+	LR-	DxAcc	PPV	NPV
ALL (n=836)	4	113	0.99 (0.98-1.0)	0.33 (0.28-0.38)	1.48 (1.37-1.59)	0.02 (0.01-0.07)	0.72 (0.69-0.75)	0.68 (0.66-0.70)	**0.97 (0.91-0.99)**
HCV (n=358)	0	34	1.0 (0.98-1.0)	0.23 (0.17-0.31)	1.30 (1.19-1.42)	0	0.68 (0.63-0.73)	0.65 (0.63-0.67)	**1.0 (0.87-1.0)**
HBV (n=27)	1	4	0.94 (0.71-1.0)	0.40 (0.12-0.74)	1.57 (0.93-2.64)	0.15 (0.02-1.14)	0.74 (0.54-0.89)	0.73 (0.61-0.82)	**0.80 (0.34-0.97)**
ALD (n=203)	0	12	1.0 (0.98-1.0)	0.35 (0.20-0.54)	1.55 (1.21-1.98)	0	0.89 (0.84-0.93)	0.89 (0.86-0.91)	**1.0 (0.70-1.0)**
NASH (n=248)	3	63	0.97 (0.91-0.99)	0.41 (0.34-0.50)	1.66 (1.45-1.91)	0.07 (0.02-0.03)	0.63 (0.57-0.69)	0.52 (0.48-0.55)	**0.96 (0.87-0.99)**

Supplementary table 4. Differences between NASH patients with LSM ≥25 kPa and with and without clinically significant portal hypertension.

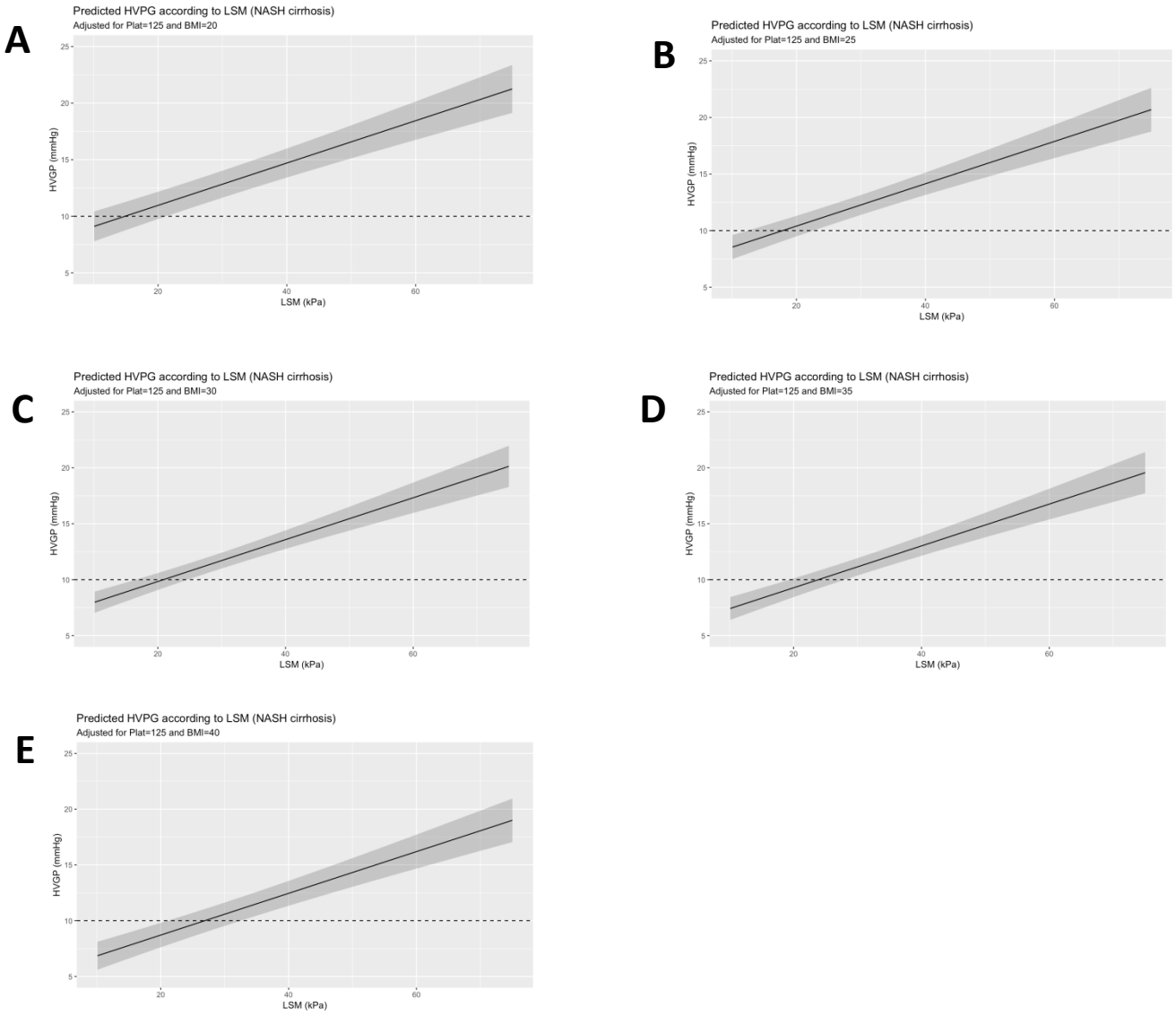
Characteristics	HVPG <10 mmHg N= 17	HVPG ≥10 mmHg N=57	P value
Age, years	56 (48-60)	61 (55-68)	**0.004**
BMI, kg/m^2	37.2 (34-40.9)	29 (25.5-34.8)	**0.024**
Platelets, x10^9/L	136 (111-250)	117 (76-162)	**0.001**
Albumin, g/L	40 (38-42)	38.7 (35-42)	0.221
Creatinine, umol/L	91.1 (88.4-109)	88.4 (88.4-88.4)	0.253
AST, IU/L	47 (31.5-83)	49 (36-62)	0.474
ALT, IU/L	52 (37-76)	38 (28-68)	**0.047**
GGT, IU/L	69 (36-224)	184 (107-348)	0.673
FIB4	2.4 (1.1-3.2)	4.1 (2.87-6.4)	**0.009**
Child-Pugh, n (%) **5** **6**	 15 (88.2) 2 (11.8)	 37 (64.9) 20 (35.1)	 0.065
MELD	7 (7-9)	9 (8-10)	**0.026**
LSM, kPa	31.9 (26.6-40.3)	45 (32.5-67.8)	**0.012**

BMI: Body mass index; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; GGT: Gamma glutamyl transferase; FIB4: Fibrosis-4 score; MELD: Model for end-stage liver disease; LSM: Liver stiffness measurement; HVPG: Hepatic venous pressure gradient.

Supplementary figure 3. Predicted probability of CSPH according to LSM by logistic regression model in non-NASH patients with obesity (BMI ≥30 kg/m^2 in orange color line) and without obesity (BMI <30 kg/m^2 in green color line).



Supplementary figure 4. Predicted mean hepatic venous pressure gradient (HVPG) value according to liver stiffness measurement (LSM) in NASH patients for A) body mass index (BMI) = 20 kg/m^2; B) BMI= 25 kg/m^2; C) BMI= 30 kg/m^2; D) BMI= 35 kg/m^2; E) BMI= 40 kg/m^2. For clarity, platelet count value was fixed at 125 x10^9



Point-by-point answers to reviewers' comments:

Editor/Editorial Board Comments:

Editors: After reviewing the manuscript and the comments from the peer reviewers, we would like to ask the authors to address the following issues raised by the editors:

1. Was there any correlation of their findings with EGD, and specifically with the absence/presence of high-risk esophageal varices warranting prophylaxis with beta-blockers or endoscopic band ligation?

This a very good point, but information regarding endoscopy was not requested to the participating centers (only LSM and HVPG were available) and it was not an objective of this study. Only the "ANTICIPATE" cohort had endoscopy data and this has been published in the "Anticipate" paper (Hepatology 2016; 64:2173–84*) and the Expanded Baveno paper (Hepatology 2017; 66:1980-8).*

2. Can the authors please convert lab values in table 1 from SI to conventional units?

Done.

3. We request the authors provide clearer instructions on how to use the nomogram from figure 3. Maybe they can provide an example or improve the instructions, something similar to what was described for the nomogram in figure 4 of the Hepatology 2016 paper on the "Anticipate" study.

Thank you for the request. We have now changed the legend of figure 3 improving the instructions to follow the nomogram (similar to the"Anticipate" study) and providing an example to make more comprehensible the calculation.

4. We acknowledge and appreciate the authors sharing the fact they had submitted the manuscript to another journal first, and especially for providing the comments from reviewers and the authors revisions. We think the quality of manuscript was improved by such revisions.

Thank you.

Reviewer #1: The authors present an excellent study which analyzed very interesting cohorts und revealed important insight especially in the emerging etiology NASH.
the two major findings in this paper on my opinion are:
1. the relationship of BMI with HVPG in NASH
2. a nomogram for the diagnosis of CSPH
the data support the conclusions and I have few comments:

1. alcoholic liver disease seems to have a more advanced disease. does this correlate

also with the degree of fibrosis? in other words, are the groups also controlled for progression of disease?

As the reviewer mentions fibrosis degree is an important component that should be considered. Alcoholic patients, despite common inclusion criteria among groups, had higher mean LSM and HVPG, and higher prevalence of PH and CSPH compared to any other etiologic group. On the other hand, when analyzing the prevalence of PH in different ranges of LSM values (Suppl Table 1), alcoholic patients presented always a higher prevalence of PH compared to other etiologies. It seems that alcoholic patients have more advanced disease even with similar LSM values. As the reviewer points out, having information regarding liver histology, in addition to LSM and HVPG, could have helped understanding whether alcoholics presented a more advanced disease or at least, higher amounts of collagen deposition. As explained, this was not the aim of our study and liver histology was not available from these patients.

2. what about the treatments the patients have received or receive? do the authors see any relationship to NSBB, statins, ACE-I, or others?

This is another important variable that might have influenced HVPH values and that it was not taken into account when collecting the data and consequently, it cannot be analyzed. A comment has been added in the study limitations regarding this issue (pag. 14, last para.)

3. what was the time between LSM and HVPG?

It was 3 months (first para., Study cohorts section of Patient and Methods).

4. HCV patients show higher AST/ALT, yet LSM and HVPG are similar, do the authors see any effect of hepatic inflammation on LSM?

All HCV patients recruited had AST/ALT values below 200 U/L and as the reviewer mentions LSM and HVPG values of HCV patients were not much different from other groups (except for alcoholic patients who had higher values). We feel that the influence of AST/ALT values on LSM values of HCV values was very little.

Reviewer #2: Overall a well written and conceptualized paper.

This is an important clinical area as identifying the cohort of Child Pugh A patients with CSPH is important for their ongoing management.

I feel the discussion could explore in more detail the potential mechanisms for your finding of reduced CSPH in the nash cohort, especially as their bmi increases. Are there any other factors at play beyond the equipment i.e. M prob or XL probe?

The reason for the lower prevalence of PH and CSPH in the obese NASH population, despite similar LSM values to other etiologies, is unknown. From the theoretical point of view this could be caused by an overestimation of LSM by elastography in these patients (with similar liver damage) or because obese NASH patient livers produce less increments of portal pressure (with similar liver damage). Basically, two potential

factors have been implicated for the first cause; the presence of liver fat and the presence of body fat. Both might be considered "technical issues" regarding interferences with the LSM readings by fat accumulation. Regarding liver fat, results are contradictory and recent studies point out to no or little influence. In relation to body fat and consequently abdominal girth, there are evidences that they might increase LSM readings. This has been discussed in our paper. LSM inaccuracy in NASH patients might be just a "technical issue" in obese people related to skin-to-liver distance or other physical characteristics or related to an interference in LSM readings caused by fat accumulation in the subcutaneous tissue. For the second mechanism (obese NASH patient livers causing less increments of portal pressure), without a very careful study with simultaneous liver biopsy (with collagen proportional area measurements), HVPG and LSM from NASH patients (obese and non-obese) it will be very difficult to have a convincing answer. In summary, liver biopsy is an important missing piece of this puzzle, and we have now acknowledged this limitation in our work in the Discussion (pag.14, end of 3rd para.).