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CLINICAL STUDIES

A novel liver stiffness measurement-based prediction model for cirrhosis in hepatitis B patients

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Keywords
aminotransferase – chronic hepatitis B – cirrhosis – liver biopsy, prediction – liver stiffness measurement

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Abstract

Backgrounds/aims: While liver stiffness measurement (LSM) predicts histological cirrhosis accurately, complementary methods are needed for better performance. Furthermore, alanine aminotransferase (ALT) influences LSM, making it necessary to modify its use in patients with high ALT levels. We developed a new LSM-based prediction model for cirrhosis and estimated the thresholds for different ALT levels. *Methods:* From 2008 to 2009, we prospectively enrolled 330 consecutive patients who were diagnosed with chronic hepatitis B (CHB) and underwent a liver biopsy and LSM on the same day. For detection of cirrhosis, we performed univariate and multivariate analyses, using the χ^2-test/*t*-test and logistic regression respectively. Thereafter, a prediction model was derived from multivariate predictors. *Results:* In multivariate analyses of patients with and without cirrhosis, we found significant differences in the LSM, spleen diameter and platelet count. Then, we developed an LSM–spleen diameter to platelet ratio index (LSPI): (LSM $\times$ spleen diameter/platelet count) $\times$ 100. The area under the receiver operating curve was 0.956, significantly higher than LSM alone (0.919, $P = 0.032$). We suggested different thresholds in patients with ALT $\leq$ upper limit of normal (ULN) (normal-ALT group, 164 patients) and ALT $>$ ULN (high-ALT group, 166 patients). In the normal-ALT group, LSPI thresholds of 38 and 62 provided 95.7% negative predictive value (NPV) and a 95.5% PPV (positive predictive value), while in the high-ALT group, thresholds of 42 and 94 yielded 95.1% NPV and 96.4% PPV respectively. Therefore, liver biopsy could be avoided in 76.7% of the subjects. *Conclusions:* LSPI is a useful, non-invasive tool that can replace liver biopsy in the assessment of liver fibrosis in the majority of CHB patients.

With current advances in antiviral treatment, maintenance of viral suppression can reduce liver-related complications in chronic hepatitis B (CHB) patients with severe liver fibrosis or early cirrhosis (1). Thus, detecting cirrhosis earlier is of paramount importance in determining whether and when to begin antiviral therapy. Liver biopsy remains the best method for assessing liver fibrosis. However, it is limited by its invasiveness, cost, risk of complications, poor acceptance, the lack of availability of expert practitioners and intra-/interobserver variability (2). These drawbacks rule out the feasibility of a liver biopsy in a sequential manner when repeated examinations are required. Currently, liver stiffness measurement (LSM), which relies on the calculation of liver elasticity from the velocity of a low-frequency elastic wave inside the liver, is extensively used as a non-invasive surrogate tool for liver biopsy to assess liver fibrosis (3–5). According to several published studies, LSM using FibroScan® was shown to have a high sensitivity and specificity in detecting histological cirrhosis (5–7).

However, despite these promising results, variations in the predictive thresholds of the LSM values among different studies and the relatively low diagnostic accuracy in patients with CHB compared with those with chronic hepatitis C (CHC) have hampered the universal application of LSM as a substitute for liver biopsy in the clinical field, especially in regions with a high prevalence

of CHB (5, 8, 9). The usefulness of LSM might also be limited by several factors such as ascites, obesity, narrow intercostal spaces, available measurement sites and other biochemical environments such as alanine aminotransferase (ALT) flare (10–13). Therefore, complementary approaches are still needed to achieve higher diagnostic accuracy as a surrogate of liver biopsy. Recently, on the other hand, LSM was reported to increase with respect to necroinflammatory activity and this has been supported by several studies in which variations in the LSM value and its diagnostic performance were observed during ALT flares in patients with chronic viral hepatitis (14–16). Thus, considering the occasional biochemical flare-up in the natural course of HBV infection, in order to prevent unnecessary overestimation of fibrosis when accompanied by ALT elevation, it is necessary to apply different LSM reference values in such a setting. For this reason, it has been suggested that non-invasive tests for various stages of liver fibrosis, such as LSM, should be analysed in the context of ALT levels and that different predictive thresholds should be used according to ALT levels (14, 15, 17). Despite this, little work has been carried out to generate a prediction model for cirrhosis using LSM in combination with other parameters reflecting progression of chronic liver disease, in order to improve the diagnostic value, especially in CHB patients.

The aims of this study were to generate a new LSM-based simple non-invasive prediction model for cirrhosis in a population with CHB and to determine and validate each different predictive threshold value optimized in the setting of a high ALT level as well as a normal ALT level respectively.

Patients and methods

Patients

Between July 2008 and August 2009, we prospectively enrolled 391 consecutive patients who had been diagnosed with CHB and who had undergone both liver biopsy and LSM on the same day, at Severance Hospital, Yonsei University College of Medicine, Seoul, Korea. The indications for liver biopsy included assessment of the severity of liver fibrosis and inflammation before treatment. The exclusion criteria were as follows: any aetiologies for chronic liver disease other than CHB, including liver cancer, co-infection with the hepatitis C virus, hepatitis D virus or human immunodeficiency virus ($n = 9$); comorbidities associated with CHB (non-alcoholic steatohepatitis, primary sclerosing cholangitis or primary biliary cirrhosis) ($n = 10$); alcohol ingestion in excess of 40 g/day for > 5 years ($n = 5$); antiviral therapy before liver biopsy ($n = 18$); previous liver resection surgery or liver transplantation ($n = 9$); unreliable LSM ($n = 5$); cardiac failure ($n = 2$); and liver biopsy unsuitable for fibrosis staging ($n = 3$). Sixty-one patients were excluded based on these criteria. Ultimately, 330 patients were included in the statistical analysis. All the patients systematically underwent complete biochemical workups, LSMs, ultrasonography and liver biopsy within 2 days.

The study protocol was in accordance with the ethical guidelines of the 1975 Declaration of Helsinki. Written informed consent was obtained from each participant or responsible family member after the possible complications of the diagnostic procedures had been fully explained. This study was approved by the Institutional Review Board of Severance Hospital.

Assessment of liver stiffness measurement and ultrasonographic evaluation and other non-invasive tests

Transient elastography was performed by one well-trained technician using the FibroScan® (Echosens, Paris, France). Details of the technique and examination procedure have been reported previously (18). The results were expressed in kilopascals (kPa). Fewer than eight successful acquisitions or a success rate of $< 60\%$ were considered unreliable.

Immediately after transient elastography, all patients underwent an ultrasonographic examination of the upper abdomen, performed by two independent experienced operators with an experience of > 10 years (K. H. Han and D. Y. Kim). A spleen bipolar diameter was defined as the greatest longitudinal dimension at the level of the splenic hilum with electronic calipers on the image monitor (19). Its measurement was technically feasible in all patients. The interobserver coefficient of variation was 1.3%, and according to the above definition, we selected the higher value of two by two operators as the spleen diameter.

All operators of the abdomen ultrasonography and transient elastography were independent from one another and blinded to the others' instrumental results and the patients' clinical and laboratory data.

Other non-invasive tests such as the AST/ALT ratio (20), age–platelet index [the sum of age factor and platelet count factor, age (years): $< 30 = 0$; 30–39 = 1; 40–49 = 2; 50–59 = 3; 60–69 = 4; $\geq 70 = 5$, platelet count (10^9/L): $\geq 225 = 0$; 200–224 = 1; 175–199 = 2; 150–174 = 3; 125–149 = 4; $< 125 = 5$] (21), the AST-to-platelet ratio index (22) [APRI, (AST/upper limit of normal) $\times$ 100/platelet count (10^9/L)], spleen-to-platelet ratio index (23) [SPRI; spleen size (cm) $\times$ 100/platelet count (10^9/L)] and age–spleen–platelet ratio index (23) (the sum of age factor and SPRI, Age (years): $< 30 = 0$; 30–39 = 1; 40–49 = 2; 50–59 = 3; 60–69 = 4; $\geq 70 = 5$) were calculated using published formulas.

Liver biopsy examination

Percutaneous liver biopsy was performed using the 16 G disposable needle. The liver biopsy specimens were fixed in formalin and embedded in paraffin. Then, 4-μm–thick sections were stained with haematoxylin and eosin and Masson's trichrome. All liver tissue samples were evaluated by a single expert hepatopathologist (Y. N. Park) with > 15 years of experience, who was blinded to the

patients' clinical histories and LSM values. Specimens that were shorter than 15 mm and considered by the pathologists to be unsuitable for fibrosis assessment were excluded from analysis. Liver fibrosis on histology was evaluated semi-quantitatively according to the METAVIR scoring system (24). Fibrosis was staged on a 0–4 scale: F0, no fibrosis; F1, portal brosis without septa; F2, portal brosis with few septa; F3, numerous septa without cirrhosis; and F4, cirrhosis.

Statistical analyses

The goals of this study were to detect the presence of histological cirrhosis using the LSM-based prediction model, which consisted of clinically relevant variables, and to suggest its different thresholds of predictive values and algorithms in patients with ALT $\leq$ upper limit of normal (ULN: defined as 45 IU/L) (normal-ALT group) and ALT $>$ ULN (high-ALT group).

In the first place, univariate analyses were performed, in order to detect candidate variables among various clinical factors for the generation of new formula distinguishing patients with histological cirrhosis (F4) from those with fibrosis ≤ 3. Differences between continuous and categorical variables were examined statistically using Student's *t*-test or the Mann–Whitney test, if appropriate, and the χ^2-test respectively. Thereafter, variables with $P < 0.05$ in the univariate analysis were included in a subsequent multivariate analysis, where logistic regression analyses were used to select variables to be maintained in the final model. Factors with $P < 0.05$ were finally selected as components of the new formula. Based on these multivariate predictors, we derived a multiple fractional equation for the prediction of cirrhosis.

To assess diagnostic accuracy, receiver operating characteristic (ROC) curves were constructed and each area under ROC curve (AUROC) was computed. Then, to evaluate the usefulness of the new predictive method in each group, the sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were calculated using ROC curves. For the comparison of the AUROC values among various tests, we used the method suggested by Hanley and McNeil (25).

To validate the model in each group and to consider its application in clinical practice, we performed an internal validation by means of the bootstrap resampling method, which involved generating ROC curves by randomly drawing 100 samples with replacement from the normal-ALT and the high-ALT group respectively. Then, the sensitivity, specificity, PPV and NPV for the suggested thresholds of predictive values were calculated in each unit (consisting of 100 samples). Thereafter, this process was repeated 1000 times. Finally, the mean values of sensitivity, specificity, PPV and NPV were computed from these 1000 units.

A probability level (P) of 0.05 was chosen for statistical significance. Statistical analyses were performed using SAS software version 9.1.3 (SAS Inc., Cary, NC, USA) and R 2.8.1 (The R Foundation for Statistical Computing, Vienna, Austria).

Results

Patients' baseline characteristics

The patients' characteristics are summarized in Table 1. The normal-ALT group included 164 patients (99 patients having F4, 60.4%) and the high-ALT group included 166 patients (80 having F4, 48.2%). There was a significant positive correlation by Spearman's correlation analysis both between necroinflammatory grade and ALT and between necroinflammatory grade and LSM. In patients with non-cirrhosis, necroinflammatory grade was significantly correlated with both ALT ($r = 0.617$, $P < 0.001$) and LSM ($r = 0.474$, $P = 0.001$). Similarly, in patients with histological cirrhosis, increasing necroinflammatory grade significantly correlated with increasing ALT ($r = 0.490$, $P < 0.001$) and LSM ($r = 0.493$, $P < 0.001$). All patients had a well-preserved liver function.

Comparisons of variables according to the presence of cirrhosis

Table 2 shows the various parameters of the patients according to the presence of cirrhosis. We observed significant differences in age ($P < 0.001$), LSM

Table 1. Baseline characteristics of the entire cohort ($n = 330$)

Variables	
Age (years)	43.7 ± 13.2
Sex (male:female)	179:151
BMI (kg/m^2)	23.4 ± 3.19
LSM (kPa)	14.8 ± 12.6
White cell count (10^9/L)	5.499 ± 2.031
Haemoglobin (mmol/L)	2.14 ± 0.27
Platelet count (10^9/L)	174.6 ± 66.0
Prothrombin time (INR)	1.05 ± 0.12
Total bilirubin (μmol/L)	17.34 ± 5.44
Albumin (g/L)	42.3 ± 5.9
AST (IU/L)	55.18 ± 17.26
ALT (IU/L)	77.00 ± 28.49
Bun (mmol/L)	4.66 ± 2.28
Creatinine (μmol/L)	85.68 ± 15.06
Spleen diameter (cm)	10.55 ± 1.97
Fibrosis stage	
F0	1, 0.3%
F1	36, 10.9%
F2	90, 27.3%
F3	24, 7.3%
F4	179, 54.2%
Necroinflammatory grades	
A1	208, 63.0%
A2	93, 28.2%
A3	29, 8.8%

Values are expressed as mean $\pm$ standard deviation.
ALT, alanine aminotransferase; AST, aspartate aminotransferase; BUN, blood urea nitrogen; INR, international normalized ratio.

Table 2. Comparisons and uni/multivariate analysis of variables to identify independent factors for predicting cirrhosis

	Patients with F $\leq$ 3 (n = 151)	Patient with F4 (n = 179)	Univariate analysis	Multivariate analysis
Variables				
Age (years)	36.42 ± 13.75	49.73 ± 9.15	< 0.001	NS
Sex (male:Female)	86:65	93:86	0.364	–
BMI (kg/m^2)	23.32 ± 3.35	23.44 ± 3.00	0.863	–
LSM (kPa)	7.53 ± 2.70	20.99 ± 14.37	< 0.001	< 0.001
White cell count (10^9/L)	6.208 ± 1.892	5.142 ± 2.012	< 0.001	NS
Haemoglobin (mmol/L)	2.22 ± 0.25	2.10 ± 0.27	0.002	NS
Platelet count (10^9/L)	218.88 ± 58.75	137.33 ± 45.7	< 0.001	0.001
Prothrombin time (INR)	1.01 ± 0.11	1.09 ± 0.12	< 0.001	NS
Total bilirubin (μmol/L)	17.00 ± 5.95	21.08 ± 4.76	< 0.001	NS
Albumin (g/L)	44.7 ± 4.3	40.4 ± 6.4	0.740	–
AST (UI/L)	63.03 ± 33.43	48.52 ± 40.70	0.079	–
ALT (UI/L)	109.9 ± 59.09	49.09 ± 32.28	0.001	NS
Bun (mmol/L)	4.37 ± 1.19	4.80 ± 2.68	0.218	–
Creatinine (μmol/L)	81.26 ± 13.25	79.50 ± 15.89	0.472	–
Spleen diameter (cm)	9.47 ± 1.35	11.47 ± 1.94	< 0.001	< 0.001

ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; INR, international normalized ratio; NS, not significant.

($P < 0.001$), white blood cell count ($P < 0.001$), haemoglobin ($P = 0.002$), platelet count ($P < 0.001$), prothrombin time ($P < 0.001$), total bilirubin ($P < 0.001$), ALT ($P = 0.001$) and spleen diameter ($P < 0.001$) between the two groups (Table 2). These univariate predictors were entered into a stepwise logistic regression model. Ultimately, LSM ($P < 0.001$; odds ratio 1.644, 95% CI 1.281–2.111), spleen diameter ($P < 0.001$; odds ratio 2.928, 95% CI 1.647–5.204) and platelet count ($P < 0.001$; odds ratio 0.984, 95% CI 0.971–0.997) were confirmed as independent predictors of cirrhosis. The AUROCs were 0.919 (95% CI 0.892–0.946), 0.803 (0.757–0.849) and 0.863 (95% CI 0.824–0.901) respectively (Fig. 1).

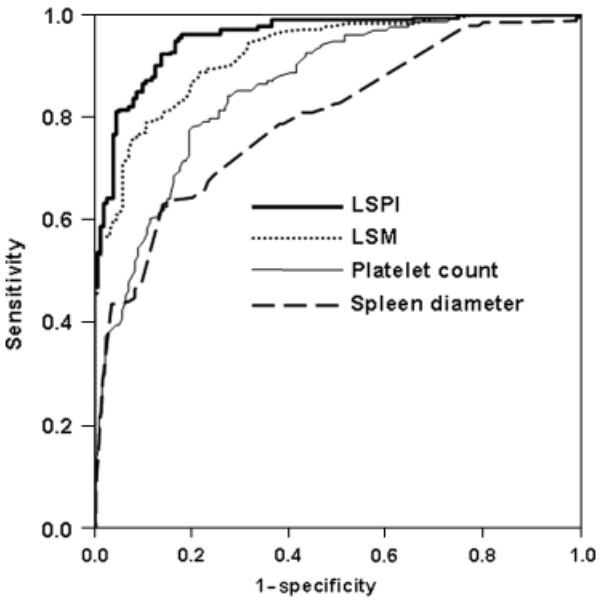

Fig. 1. Receiver-operating characteristics (ROC) curve of the LSM–spleen diameter to platelet ratio index (LSPI), liver stiffness measurement (LSM), spleen diameter and platelet count for the diagnosis of cirrhosis.

Formula for prediction of cirrhosis and comparisons of diagnostic accuracy among tests

Based on the above multivariate analysis, we derived a multiple fractional equation for the prediction of cirrhosis that included LSM (odds ratio > 1) and spleen diameter (odds ratio > 1) as the numerator and platelet count (odds ratio < 1.0) as the denominator, in order to amplify the effect of each factor on the progression of fibrosis. Therefore, we propose the following model, which we call LSPI (LSM–spleen diameter to platelet ratio index): (LSM × spleen diameter/platelet count) × 100. The AUROC of LSPI was 0.956 (95% CI 0.936–0.976), showing the superior diagnostic accuracy from using multiple factors over a single factor LSM ($P = 0.032$), spleen diameter ($P < 0.001$) and platelet count ($P < 0.001$). Furthermore, we observed a better AUROC result of LSPI, as compared with other non-invasive tests such as the AST/ALT ratio (20) (0.716, 95% CI 0.660–0.771; $P < 0.001$), age–platelet index (21) (0.875, 95% CI 0.838–0.912; $P < 0.001$), APRI (22) (0.685, 95% CI 0.626–0.744; $P < 0.001$), spleen to platelet ratio index (23) (0.902, 95% CI 0.869–0.934; $P = 0.006$) and age–spleen–platelet ratio index (23) (0.916, 95% CI 0.887–0.945; $P = 0.026$). The AUROC of LSPI was also statistically higher than that of the simpler formula, that is, the LSM to platelet ratio index [defined as (LSM/platelet count) × 100, 0.936 (95% CI 0.904–0.960), $P = 0.012$].

In the whole entire population, we firstly provided the lower and upper predictive thresholds in order to obtain at least 95% of NPV and PPV for cirrhosis. Between two points, we identified a 'grey zone,' within which lie

Table 3. Suggested predictive threshold values of the LSM–spleen diameter to platelet ratio index for the prediction of cirrhosis in each group

	LSPI	NPV (%)	PPV (%)	Sensitivity (%)	Specificity (%)
Normal ALT level					
Lower limit (at least 95% NPV)	38	95.7 (84.2–99.2)	82.9 (74.5–89.0)	98.0 (92.1–99.6)	69.2 (56.4–79.7)
Upper limit (at least 95% PPV)	62	81.3 (70.3–89.0)	95.5 (88.2–98.5)	85.9 (77.0–91.7)	93.8 (84.2–98.0)
High ALT level					
Lower limit (at least 95% NPV)	42	95.1 (85.4–98.7)	73.3 (63.6–81.2)	96.3 (88.6–99.0)	67.4 (56.3–76.9)
Upper limit (at least 95% PPV)	94	76.4 (67.1–83.7)	96.4 (86.6–99.3)	67.5 (55.9–77.3)	97.7 (91.0–99.5)

In parentheses, 95% confidence interval.

ALT, alanine aminotransferase; LSPI, LSM–spleen diameter to platelet ratio index; NPV, negative predictive value; PPV, positive predictive value.

Table 4. Validation of the suggested predictive threshold values of the LSM–spleen diameter to platelet ratio index for the prediction of cirrhosis in each group from bootstrap samples

	LSPI	NPV (%)	PPV (%)	Sensitivity (%)	Specificity (%)
Normal ALT level					
Lower limit (at least 95% NPV)	38	95.8 (83.8–99.3)	82.8 (73.1 -90.2)	98.0 (92.1–99.7)	68.8 (53.8–81.4)
Upper limit (at least 95% PPV)	62	81.3 (68.7–90.5)	95.5 (87.8–98.8)	85.9 (75.8–92.9)	93.8 (83.6–98.3)
High ALT level					
Lower limit (at least 95% NPV)	42	95.1 (84.8–98.9)	73.3 (61.9–83.0)	96.3 (88.3–99.2)	67.3 (54.1–78.8)
Upper limit (at least 95% PPV)	94	76.4 (65.5–85.3)	96.4 (86.0–99.4)	67.4 (53.8–79.2)	97.6 (90.7–99.6)

In parentheses, 95% CI.

ALT, alanine aminotransferase; LSPI, LSM–spleen diameter to platelet ratio index; NPV, negative predictive value; PPV, positive predictive value.

equivocal cases where a liver biopsy is needed. At the threshold of 39 (105 patients), an NPV of 96.1% (95% CI 90.5–98.9%), a PPV of 77.8% (95% CI 71.8–83.0%), a sensitivity of 97.8% (95% CI 94.4–99.4%) and a specificity of 66.9% (95% CI 58.8–74.3%) were achieved, whereas at the threshold of 77 (142 patients), an NPV of 77.1% (95% CI 70.5–83.0%), a PPV of 95.8% (95% CI 91.0–98.4%), a sensitivity of 75.9% (95% CI 69.0–82.0%) and a specificity of 96.0% (95% CI 91.6–98.5%) were provided. Overall, 74.8% of the population might have avoided an unnecessary liver biopsy.

Determination and validation of optimal predictive threshold values of the LSM–spleen diameter to platelet ratio index for the detection of cirrhosis according to the alanine aminotransferase level

Because LSM and LSPI tend to increase with the ALT level, we estimated different predictive threshold values for LSPI that can be used for the accurate prediction of cirrhosis in two groups: the normal-ALT and the high-ALT groups. Our major goal was to determine the optimal predictive thresholds in each subset at which histological cirrhosis can be accurately excluded or diagnosed without performing a liver biopsy. Similarly, in each group, we proposed two predictive threshold values, the lower and upper points, which were chosen in order to obtain at least 95% of NPV and PPV.

Table 3 shows the low and high predictive threshold values of LSPI for the prediction of cirrhosis according to the ALT level. In the normal-ALT group, an LSPI predictive threshold value of 38 (47 patients) had an NPV of 95.7 (95% CI 84.2–99.2%), while an LSPI predictive threshold value of 62 (89 patients) had a PPV of 95.5% (95% CI 88.2–98.5%). Hence, 82.9% of the patients in the normal-ALT group might have avoided an unnecessary liver biopsy. Likewise, in the high-ALT group, when LSPI was set at 42, an NPV 95.1 % (95% 85.4–98.7%) was observed in 61 patients, whereas an LSPI predictive threshold of 94 resulted in a PPV of 96.4% (95% CI 86.6–99.3%) in 56 patients. As a result, liver biopsy might have been avoided in 70.5% of the patients in the high-ALT group. However, patients within the 'grey zones,' that is, LSPI 38–62 in the normal-ALT group or LSPI 42–94 in the high-ALT group, should still undergo a liver biopsy for accurate assessment of fibrosis. The above diagnosis interval by LSPI in the normal-ALT group (82.9%) was significantly broader than that in the high-ALT group (70.5%) ($P = 0.008$).

The results of the above analyses were validated using the bootstrap resampling method. The results of the internal validation are shown in Table 4. There is a very good agreement between the results obtained from original and bootstrap samples. For the prediction of cirrhosis in the normal-ALT group, an LSPI predictive threshold value of 38 resulted in an NPV of 95.8% (95% CI 83.8–99.3%) and an LSPI predictive threshold value of 62 resulted in a PPV of 95.5 % (95% CI 87.8–98.8%). Similarly, in the high-ALT group, an NPV of 95.1% (95% CI 84.8–98.9%) was achieved for an LSPI predictive threshold of 42, while a PPV of 96.4% (95% CI 86.0–99.4) was obtained with an LSPI predictive

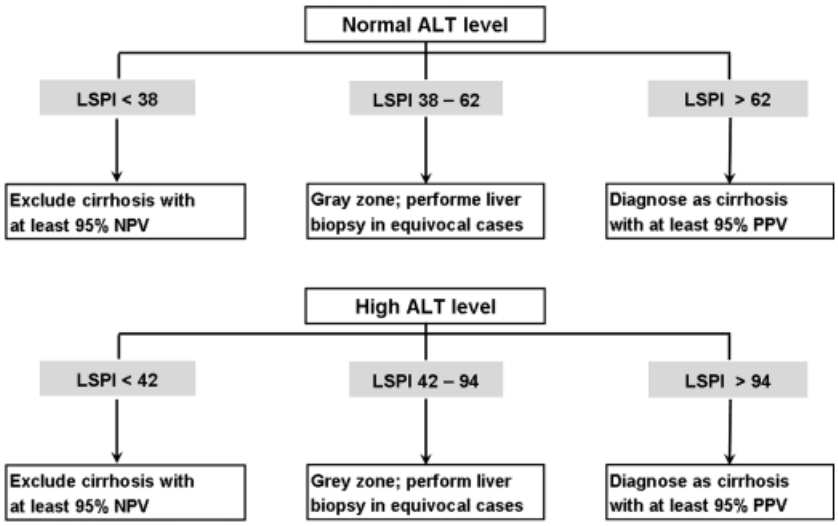

Fig. 2. Proposed management algorithm for the diagnosis of cirrhosis according to different alanine aminotransferase (ALT) levels.

threshold of 94. Therefore, we proposed a new management strategy algorithm (Fig. 2).

Discussion

Early diagnosis of cirrhosis is an important clinical issue, as it is a pivotal factor in determining a treatment plan for antiviral therapy and predicting the long-term clinical course in patients with CHB, and yet, early non-invasive diagnosis of cirrhosis is difficult to perform because liver function and ultrasonographic findings are grossly normal. Liver biopsy remains the gold standard for assessing liver fibrosis; however, it has the disadvantages of invasiveness, cost, poor compliance and the lack of available expertise (2). Although LSM has considerable accuracy in the prediction of fibrosis, vast differences in the diagnostic power and predictive thresholds among studies are obstacles to its universal application as a reliable alternative to a biopsy in the clinical field. Furthermore, microscopic conditions in active hepatitis, such as extensive inflammatory infiltration, hepatocyte swelling and tissue oedema, may represent important confounding factors in the diagnostic performance of LSM and determination of its corresponding predictive thresholds, as Fung *et al.* (16) also reported a direct correlation between LSM and biochemical parameters (14–17). Likewise, in the current study, the close correlation of the higher LSM with the higher ALT level was observed in the same histological fibrosis status. One possible theoretical background for this phenomenon may be that a liver surface may be stiffer because of surrounding tissue oedema during active inflammation and that LSM using the elastic shear wave propagation may be consequently overestimated in such a setting (14–16). Therefore, some modifications in the clinical application of LSM, including combining other fibrosis parameters with LSM, are required to improve the predictive power of LSM as a substitute for liver biopsy (14, 15, 17). Recently, Boursier *et al.* (26) suggested adding a Fibrometer® to LSM for improved diagnostic accuracy. Here, we aimed to generate a new LSM-based model by combining other clinical parameters reflecting progression of chronic liver disease and to determine each predictive threshold value optimized in the setting of a high ALT level as well as a normal ALT level respectively.

Although there have recently been several reports regarding the combination of LSM with indexes derived from other serum markers and the effects of ALT levels on LSM, our study is unique for several reasons (3, 8, 14, 15, 17). Very few investigations have been conducted to test the utility of combining LSM with other clinical variables that proved to be significant in a study population. Furthermore, to our knowledge, this is the first study defining different optimal predictive threshold values for an LSM-based model with respect to different necroinflammatory statuses. These results may help prevent the inappropriate overestimation of hepatic fibrosis. The implementation of a simple LSM-based non-invasive model and suggestion of different predictive thresholds considering each necroinflammatory status would help physicians identify patients with early cirrhosis more accurately, who require further treatments and more vigorous surveillance. Secondly, a relatively large number of consecutive subjects from a single centre were prospectively enrolled. Thirdly, in contrast to previous studies, the distribution of our study population was homogeneous and representative of the B-viral hepatitis patients seen in clinical practice. Previous studies have focused primarily on populations with CHC or heterogeneous causes (3, 15) Considering the differences in predictive threshold values and the efficacies of LSM according to the underlying aetiology, this study may

provide more useful results because it is based on patients with B-viral hepatitis.

Using multivariate analysis, we observed significant differences in the LSM, spleen diameters and platelet counts between patients with and without cirrhosis. Liver stiffness is a function of the fibrotic transformation of the liver tissue and LSM has been accepted as a highly accurate method for predicting liver fibrosis and cirrhosis (4). Spleen size has been regarded as an important clinical characteristic of chronic liver disease. Aubé *et al.* (27) proposed that spleen size was associated with portal hypertension caused by fibrotic changes of the liver and that ultrasonographic findings, including spleen size, could provide the diagnosis of cirrhosis in up to 82–88% of cases, and even in cases of clinically compensated liver disease. On the other hand, thrombocytopaenia has been reported with the progression of fibrosis in previous studies (28, 29). Increasing fibrosis and worsening portal hypertension leads to increased sequestration and destruction of platelets in the enlarged spleen. In addition, fibrosis progression is associated with decreased production of thrombopoietin by hepatocytes, resulting in reduced platelet production (30). In agreement with these hypotheses, LSM, spleen diameter and thrombocytopaenia have considerable diagnostic power, even when applied alone. The correlation of fibrosis with either spleen diameter or thrombocytopaenia could also be observed in that the AUROC of the age–spleen–platelet ratio index (0.916), one of the conventional fibrosis tests, is considerably high and similar to that of the novel test, LSM (0.919). Through integrating LSM, spleen diameter and platelet count in a ratio, we derived a new formula, LSPI. LSPI has excellent diagnostic accuracy in terms of AUROC, showing statistically significant superiority over both each factor alone and previously published non-invasive tests, whereas a few LSM-based combination models from other studies did not show a similar superiority (3, 8, 20–23, 26). The strengths of LSPI can be explained by such a pathophysiological basis underlying its conception. However, regarding the effect of ALT on cirrhosis, this study did not show a significant influence, although several non-invasive tests for cirrhosis included ALT level as their component (20, 31, 32). If ALT had an independent influence, the ALT level itself might have been introduced into the predictive model.

In the normal-ALT and high-ALT groups, exclusion of cirrhosis could be identified with a high NPV of 95.7% and 95.1% at predictive threshold values of LSPI 38 and 42 respectively. Therefore, LSPI can be reliably applied with a high accuracy to exclude cirrhosis. Likewise, the presence of cirrhosis could be predicted at a high PPV of 95.5 and 96.4% with a predictive threshold of LSPI 62 and 94 in the normal-ALT and high-ALT groups respectively. Overall, the likelihood of histological cirrhosis was correctly diagnosed with a non-invasive method in 76.7% of the subjects, which meant that an unnecessary liver biopsy could have been prevented in these patients. Patients in the border zone (those with $38 < \text{LSPI} < 62$ in the normal-ALT group or with $42 < \text{LSPI} < 94$ in the high-ALT group) would still need a liver biopsy. Instead of using a single optimal predictive thresholds, we attempted to suggest two predictive threshold points (the lower and upper limit) in each group, which could represent a high accuracy with at least 95% of NPV and PPV, respectively, because the major goal of this study was to identify the lowest and the highest risk patients who do not require a liver biopsy. Accordingly, we allowed for a grey zone, where the presence or absence of cirrhosis could not be identified with LSPI, and we recommend a liver biopsy for patients in this group. Although a single cut-off is easier to use, it may result in more cases of false negatives or false positives inherently. Based on these results, we propose a management strategy algorithm with reference to the current guidelines, in order to help physicians make clinical decisions about diagnosis and surveillance. In addition to the excellent diagnostic value of LSPI, it has several advantages in the clinical field. From a practical point of view, it is easy to calculate LSPI at bedside or in the outpatient clinic, while from a technical point of view, LSM and spleen diameter can easily be assessed with high reproducibility and low intra-/interobserver variability (10, 33, 34). Especially, the rate of obtaining a reliable LSM was higher than 98% in this study and the measurement of spleen size was technically feasible in all patients. Recently, Lucidarme *et al.* (35) reported that IQR/M is a factor of overestimation of liver brosis (F0–2 vs. F3–4) in European populations with CHC. In order to test the effect of IQR/M on LSM accuracy in our subjects, we compared the IQR/M between those patients with discordance and non-discordance, yielding no significant difference. Therefore, the validation of the effects of IQR/M in Asian populations with CHB should be performed in a further study with a higher sample size. Furthermore, from a financial viewpoint, spleen size can be measured without additional cost during routine follow-up, because ultrasonography is frequently used for periodic screening for hepatocellular carcinoma.

Bootstrap resampling analysis was used to internally validate our proposed model in the current study. This methodology was recently proposed as a breakthrough method for the internal validation of surgical regression models (36). The main advantage of this technique is that the entire dataset can be used for building a more robust model, especially in moderate-size databases and for rare outcomes (37). Furthermore, the predictive validity of the model can be assessed not only in one randomly split set of patients as in the traditional training-and-validation method but also typically in 1000 new different samples obtained by means of resampling with replacement. We observed a very good agreement between the results obtained from the original and the bootstrap samples.

This study had several limitations. Firstly, several investigators reported the improved diagnostic usefulness of combining LSM with a FibroTest® or a

Fibrometer® for liver fibrosis or cirrhosis in patients with CHC (26, 38, 39). However, as FibroTest® and Fibrometer® are rarely available in Korea, we could not evaluate this model in our subjects with CHB. Secondly, our population had a high proportion of cirrhosis. Relatively more patients with advanced fibrosis stages, including cirrhosis, tend to visit our hospital, as Severance hospital is one of the largest medical centres, as a tertiary referral hospital in Korea. Therefore, because it might, to some extent, have a detection bias, another independent external validation study in a population with a lower prevalence of cirrhosis should be conducted, in order to provide more generalizable results in patients with CHB-related chronic liver disease. Thirdly, because an invasive liver biopsy to determine the degree of fibrosis and inflammation is in fact not so necessary for overt decompensated cirrhotic patients, all the enrolled subjects had compensated cirrhosis or normal liver function. Thus, LSPI might not be reliable in more severe patients and another non-invasive method covering all the patients with chronic liver disease is required. In addition, for LSPI to be applied universally as a surrogate marker, another external validation in an independent population is required, other that the internal resampling method. Finally, because LSM was significantly correlated with the ALT level as a quantitative variable, how to adjust the ALT level in the formula or how to stratify subjects using the ALT level, besides dividing them into two groups, still remains to be established, although several investigators used a method similar to ours, dividing them into two or three groups (14, 15). Thus, more sophisticated ways are required in the future study.

In summary, using a prospective, large-scale study, we defined a new formula, LSPI, which demonstrated a better diagnostic accuracy in predicting histological cirrhosis. Based on our results, we found that biopsies might be avoided with a high certainty in the low- and high-risk group, in both normal and high ALT levels. We hope that other researchers will evaluate the reproducibility of the LSPI for the non-invasive diagnosis of cirrhosis in an independent population with different clinical backgrounds.

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