Risk assessment of hepatitis B virus-related hepatocellular carcinoma development using liver stiffness measurement (FibroScan®)

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Risk assessment of hepatitis B virus-related hepatocellular carcinoma development using liver stiffness measurement (FibroScan®)

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This certifies that the Master's Thesis
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ABSTRACT

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Background/ Aims: Liver stiffness measurement (LSM) using FibroScan® accurately assesses the degree of liver fibrosis and the risk of hepatocellular carcinoma (HCC) development in patients with chronic hepatitis C (CHC). This study investigated the usefulness of LSM as a predictor of HCC development in patients with chronic hepatitis B (CHB).

Methods: A total of 1,130 patients with non-biopsy proven CHB who underwent LSM between May 2005 and December 2007 were enrolled in this prospective study. After LSM was performed, patients attended regular follow-up as part of a surveillance program for the detection of HCC.

Results: The mean age of the patients (767 men, 363 women) was 50.2 years and the median LSM was 7.7 kPa. Six hundred seventy two (59.5%) patients received antiviral treatment before or after enrollment. During the follow-up period (median, 30.7 months; range 24.0-50.9 months), HCC developed in 57 patients (2.0% per 1 person-year). The 1, 2, and 3-year cumulative incidence rates of HCC were 0.80%, 3.26%, and 5.98%, respectively. On multivariate analysis, together with old age, male gender, heavy alcohol consumption (> 80 g/day), serum albumin, and hepatitis B e antigen positivity, patients with a

higher LSM value were at a significantly greater risk of HCC development, with a hazard ratio of 3.07 [95% CI, 1.01–9.31; P=0.047] when LSM value 8.1–13 kPa, 4.68 [95% CI, 1.40–15.64; P=0.012] when LSM value 13.1–18 kPa, 5.55 [95% CI, 1.53–20.04; P=0.009] when LSM value 18.1–23 kPa, and 6.60 [95% CI, 1.83–23.84; P=0.004] when LSM value >23 kPa, as compared to LSM value ≤8 kPa.

Conclusion: Our data suggest that LSM could be a useful predictor of HCC development in patients with CHB.

Key words : Chronic hepatitis B - Fibroscan – Hepatitis B virus – Hepatocellular carcinoma - Liver stiffness measurement - Transient elastography

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I. INTRODUCTION

Hepatocellular carcinoma (HCC) is one of the most common malignant tumors in the world. As the incidence of HCC is increasing, it is becoming a major public health problem.[1] Unless HCC is diagnosed at an early stage, poor prognosis is expected due to limited treatment options.[2] Thus, early detection of HCC is important for high-risk patients, such as those with chronic hepatitis B (CHB) and hepatitis C (CHC) infections or non-viral cirrhosis, and those exposed to environmental toxins.[3] In particular, in patients with CHB and CHC, advanced liver fibrosis and cirrhosis are significantly correlated with the risk of HCC development.[4,5] Therefore, reliable methods for the early identification of liver fibrosis progression and compensated liver cirrhosis are an essential part of an efficient surveillance program for the detection of HCC.[6]

To date, liver biopsy (LB) had been the gold standard for assessing the severity of liver fibrosis and cirrhosis.[7] Although LB is generally accepted to be a safe procedure, it can cause discomfort and carries a small risk of severe complications.[8] Furthermore, LB is prone to sampling error as only 1/50,000 of the liver is analyzed microscopically.[9] In addition, LB is not a suitable method for assessing the degree of liver fibrosis in a sequential manner only for the purpose of evaluating the risk of HCC development.

Recently, liver stiffness measurement (LSM) using FibroScan® has been introduced; it has proven clinical accuracy for the detection of liver fibrosis and cirrhosis and provided reproducible and reliable results.[10,11] Furthermore, LSM can be expressed numerically as continuous variables, which allow clinicians to grade the degree of liver cirrhosis and assess the risks of developing liver-related complications. Because of these advantages, the role of LSM is now being extended as a predictor of HCC development in patients with chronic liver disease (CLD). Masuzaki *et al*. identified an association between LSM and the presence of HCC in patients with CHC in a cross-sectional study and they showed that LSM could be used as a predictive tool for HCC development in patients with CHC in a follow-up prospective study.[12,13]

In previous cross-sectional studies, we reported different LSM values in patients having CHB with and without HCC.[14,15] However, prospective studies investigating the role of LSM as a predictor of HCC development in patients with CHB are limited. In this study, we evaluated the usefulness of LSM for assessing the risk of HCC development in a large cohort of patients with CHB.

II. MATERIALS AND METHODS

1. Patients

From May 2005 to December 2007, a total of 1,229 patients with CHB visited the liver unit of Shinchon Severance Hospital, Yonsei University College of Medicine, Seoul, Korea. CHB was defined as persistent presence of serum hepatitis B virus surface antigen (HBsAg) for more than 6 months. Patients who provided informed consent received LSM and were consecutively enrolled in this prospective study.

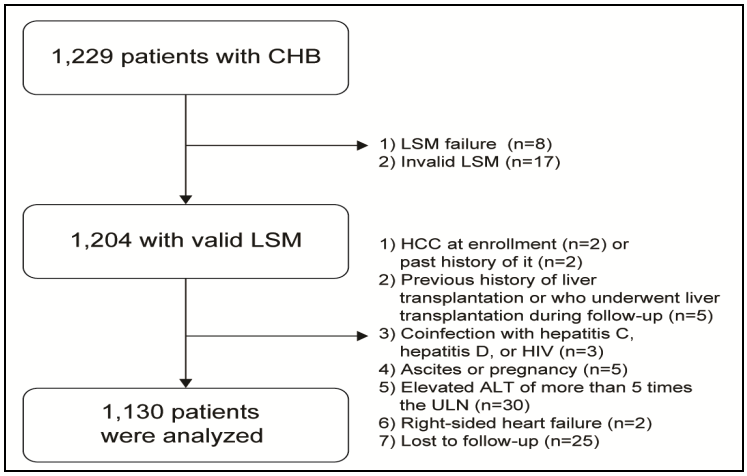
With our exclusion criteria (**Figure 1**),[16-19] 99 patients were excluded and the remained 1,130 patients were selected for statistical analysis. Twenty five patients who were excluded due to LSM failure (n=8) or an invalid LSM (n=17) had significantly higher body mass index than the other patients (28.5 *vs*. 23.7 kg/m^2, P<0.001), whereas the other variables did not differ significantly (all P>0.05, data not shown).

On the same day as LSM, blood parameters including serum albumin, total bilirubin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), prothrombin time, platelet count, and alpha-fetoprotein (AFP) were recorded. HBsAg and hepatitis B e antigen (HBeAg) were measured using standard enzyme-linked immunosorbent assays (Abbott Diagnostics, Abbott Park, IL, USA). HBV DNA levels were assessed with a hybridization capture assay (Digene Diagnostics, Gaithersburg, MD, USA) having a detection limit of 141,000 copies/mL.

If histologic information was not available, clinically diagnosed liver cirrhosis (cLC) was defined as followings: 1) the patients had a platelet count <100,000/uL and ultrasonographic findings suggestive of cirrhosis including a blunted, nodular liver edge accompanied by splenomegaly (>12 cm), 2) esophageal or gastric varices existed, or 3) overt complications of liver cirrhosis

were observed, including ascites, variceal bleeding, and hepatic encephalopathy.[20,21] The study protocol conformed to the ethical guidelines of the 1975 Helsinki Declaration and was approved by the institutional review board of our institute.

Figure 1. The recruitment algorithm.



2. Follow-up

Each patient was screened for HCC with ultrasonography at their initial visit. Two patients were excluded due to presence of HCC at initial visit. If no evidence of HCC was detected, patients were followed up with AFP and ultrasonography every 3 or 6 months. During the surveillance, HCC was diagnosed based on the guideline of American Association for the Study of Liver Diseases.[2] Briefly, patients were diagnosed with HCC if they had a tumor with a maximum diameter of >2 cm and the typical features of HCC on dynamic computed tomography (CT) (defined as hyperattenuation in the arterial phase and early washout in the portal phase), and an AFP >200 ng/ml.[2] If the maximum diameter of tumor was 1 to 2 cm, dynamic CT and magnetic

resonance imaging were performed. HCC was diagnosed if coincidental typical features of HCC were noted. If the tumor did not satisfy above criteria, a biopsy was performed. When the tumor was <1 cm, ultrasonography was repeated after 3 months. The last follow-up date in this study was December 2009.

3. Liver stiffness measurement

LSM was performed on the right lobe of the liver through the intercostal spaces on patients lying in the dorsal decubitus position with the right arm in maximal abduction.[16] The operator located a liver portion that was at least 6 cm thick and free of large vascular structures, and pressed the probe button to commence the measurement. Only one experienced technician (more than 1,000 examinations) blind to the clinical data of patients, was allowed to perform LSM. The results are expressed as kilopascals (kPa). In this study, only LSM examinations with at least 10 validated measurements and a success rate of at least 60% were considered reliable. The median value of successful measurements was selected as a representative of the LSM value in a given patient only if an interquartile range to median value ratio was less than 0.3. Any LSM value that did not satisfy the above conditions was considered unreliable and excluded from further analysis.

4. Cutoff values for liver cirrhosis and stratification interval

Initially, we adopted 13 kPa as the cutoff value for liver cirrhosis based on a previous meta-analysis[22]. Then, we adopted the same stratification interval (5 kPa) as a Japanese study with CHC[12] to stratify patients with LSM value >13 kPa, because we planned to compare the risk of HCC development between CHB and CHC. However, given that almost 80% of the study population (n=888) had LSM value ≤13 kPa, we extended our stratification below the

cutoff of liver cirrhosis by same interval. Ultimately, our study population was stratified into five groups as ≤8 kPa, 8.1–13 kPa, 13.1–18 kPa, 18.1–23 kPa, and >23 kPa.

4. Statistical analysis

Data are expressed as the means ± standard deviation, median (range), or n (%) as appropriate. When comparing the baseline characteristics of patients with and without HCC development and those with and without cLC, the chi-square test and Fisher's exact test were used for categorical data, and Student's t-test and the Mann-Whitney U test were used for continuous variables. The annual incidence rates of HCC were expressed as the person-year method. The cumulative incidence rates of HCC were calculated using the Kaplan-Meier method. The proportions of patients with HCC development and cLC according to LSM stratification were compared with Mantel-Haenszel tests. The incidence of HCC according to LSM change was compared using the chi-square test (Fisher's exact test) with the Bonferroni correction. To estimate independent risk factors for HCC development, univariate and subsequent multivariate Cox proportional hazard regression analysis were used. Hazard ratios and corresponding 95% confidence interval (CI) are indicated. A *P*-value <0.05 on two-tailed test was considered significant. Data analysis was performed using the SAS program (9.1ver, SAS Inc, North Carolina).

III. RESULTS

1. Baseline characteristics

The baseline characteristics of 1,130 patients at enrollment are summarized in **Table 1**. The mean age of our study population (767 men and 363 women) was 50.2 years. One hundred ninety-seven (17.4%) patients had cLC (178 patients with thrombocytopenia (<100,000/uL) and ultrasonographic findings suggestive of cirrhosis, nine with esophageal or gastric varices, one with overt complication of cirrhosis, and nine with more than two positive findings for cirrhosis) and most of them (n=185, 93.9%) belonged to Child-Turcotte-Pugh class A. The baseline characteristics of the patients with cLC were compared to those without (**Table 2**). At enrollment, 444 (39.3%) patients had a previous or an ongoing use of an antiviral agent (lamivudine [n=306], adefovir [n=114], entecavir [n=14], and combination of lamivudine and adefovir [n=10]), whereas 228 (20.2%) patients received antiviral treatment after enrollment (lamivudine [n=98], adefovir [n=15], and entecavir [n=115]). The median LSM value was 7.7 kPa (range 2.9-75 kPa).

Table 1. Baseline Characteristics (n = 1,130)

Variables	
Demographic data	
Age (years)	50.2 ± 9.9 (21-81)
Male	767 (67.9)
Alcohol consumption >80 g/day	59 (5.2)
Clinically diagnosed liver cirrhosis	197 (17.4)
Diabetes mellitus	41 (3.6)
Previous or ongoing antiviral treatment at enrollment	444 (39.3)
Body mass index (kg/m^2)	
Underweight (<18.5)	32 (2.8)
Normal (18.5-25)	752 (66.5)
Overweight (25.1-30)	325 (28.8)
Obesity (>30)	21 (1.9)
Laboratory results	
Serum albumin (g/dL)	4.5 ± 0.4 (2.4-6.3)
Total bilirubin (mg/dL)	0.9 ± 0.7 (0.2-4.8)
Aspartate aminotransferase (IU/L)	33.8 ± 18.2 (4-167)
Alanine aminotransferase (IU/L)	38.8 ± 27.2 (6-176)
Prothrombin time (%)	92.1 ± 12.0 (35.2-100)
Platelet count (10^9/L)	162.4 ± 64.1 (26-526)
Alpha-fetoprotein (ng/mL)	3.07 (0.5-147)
HBeAg positivity	408 (36.1)
Detectable HBV-DNA†	361 (31.9)
Liver stiffness measurement	
Liver stiffness measurement value (kPa)	7.7 (2.9-75)
Interquartile range (kPa)	1.7 ± 2.4 (0.1-9.7)
Success rate (%)	95.6 ± 8.3 (65-100)

Variables are expressed as mean ± SD, median (range), or n (%).
Lower limit of detectable HBV-DNA† was 141,000 copies/mL.
HBeAg, hepatitis B e antigen; kPa, kilopascal.

Table 2. Baseline characteristics of Patients With and Without clinically diagnosed Liver cirrhosis and HCC development

Variables	Patients with clinically diagnosed cirrhosis (n=197, 17.4%)	Patients without clinically diagnosed cirrhosis (n=933, 82.6%)	*P* value	Patients with HCC (n=57, 5.0%)	Patients without HCC (n=1,073, 95.0%)	*P* value
Demographic data						
Age (years)	54.0±8.9	49.4±9.9	<0.001	55.5±9.3	49.9±9.9	<0.001
Male	137 (69.5)	630 (67.5)	0.322	49 (86.0)	718 (66.9)	0.002
Alcohol consumption >80 g/day	18 (9.1)	41 (4.4)	0.012	11 (19.3)	48 (4.5)	<0.001
Clinically diagnosed liver cirrhosis	-	-		28 (49.1)	169 (15.8)	<0.001
Diabetes mellitus	17 (8.6)	24 (2.6)	<0.001	7 (12.3)	34 (3.2)	0.003
Previous or ongoing antiviral treatment at enrollment	81 (41.2)	363 (38.9)	0.357	19 (33.3)	425 (39.6)	0.336
Body mass index (kg/m^2)			0.711			0.947
Underweight (<18.5 kg/m^2)	1 (0.5)	31 (3.3)		2 (3.5)	30 (2.8)	
Normal range (18.5-25 kg/m^2)	138 (70.1)	614 (65.8)		38 (66.7)	714 (66.5)	
Overweight (25.1-30 kg/m^2)	54 (27.4)	271 (29.0)		15 (26.3)	310 (28.9)	
Obesity (>30 kg/m^2)	4 (2.0)	17 (1.8)		2 (3.5)	19 (1.8)	
Laboratory data						
Serum albumin (g/dL)	4.2±0.6	4.5±0.3	<0.001	4.1±0.5	4.5±0.4	<0.001
Total bilirubin (mg/dL)	1.3±0.6	0.8±0.7	<0.001	1.0±0.5	0.9±0.7	0.355
Aspartate aminotransferase (IU/L)	41.0±19.0	32.4±17.7	<0.001	43.0±20.5	33.4 ± 17.9	<0.001
Alanine aminotransferase (IU/L)	39.1±21.0	38.8±28.4	0.856	44.0±25.0	38.5 ± 27.4	0.145
Prothrombin time (%)	85.1±13.7	94.5±10.4	<0.001	86.0±14.1	92.7±11.7	0.001
Platelet count ($10^9/L$)	95.9±36.9	180.4±57.8	<0.001	129.2±49.8	164.6 ± 64.4	<0.001
Alpha-fetoprotein (ng/mL)	16.8	5.5	<0.001	34.5	6.0	0.016
HBeAg positivity†	72 (37.3)	336 (36.6)	0.457	28 (49.1)	380 (35.4)	0.047
Detectable HBV-DNA	70 (35.5)	291 (31.2)	0.129	24 (42.1)	337 (31.4)	0.106
Liver stiffness measurement						
Liver stiffness measurement values (kPa)	17.3	6.8	<0.001	16.1	7.5	<0.001

Variables are expressed as mean ± SD, median (range), or n (%).HCC, hepatocellular carcinoma; HBeAg, hepatitis B e antigen; kPa, kilopascal.

2. The Incidence, Stage, and Treatment of HCC

The median follow-up period was 30.7 months (range 24.0–50.9 months) constituting 2,885 person-years overall. HCC developed in 57 patients (2.0% per 1 person-year) during the study period. The cumulative incidence rates of HCC at 1, 2, and 3 years were 0.80%, 3.26%, and 5.98%, respectively. No significant difference existed in the duration of follow-up between patients with HCC development and those without (31.5 *vs*. 29.5 months, *P*=0.126). According to the staging system of Liver Cancer Study Group of Japan[23], 33 (57.9%) patients belonged to stage I, 16 (28.1%) to stage II, and 8 (14.0%) to stage III. Hepatic resection was done for 42 (73.7%) patients and radiofrequency ablation for 5 (8.8%) with curative aims. Palliative treatments including transarterial chemoembolization (n=6, 10.5%) and intra-arterial chemotherapy (n=4, 7.0%) were also performed.[24] HCC was confirmed histologically in 42 patients with hepatic resection.

3. Comparison between patients with HCC development and those without

The clinical characteristics at enrollment between patients with HCC development and those without are compared in **Table 2**. Age, the proportion of males, heavy alcohol consumption (>80 g/day), the proportions of cLC and diabetes mellitus, AST, AFP, HBeAg positivity, and LSM value were significantly higher among patients with HCC development, whereas serum albumin, prothrombin time, and platelet count were significantly higher among those without (all P<0.05). Among the 57 patients with HCC, esophageal or gastric varices were found at enrollment in eight (14.0%) patients and no other liver-related complication was found at enrollment.

4. Risk Analysis of HCC Development According to LSM value

The proportion of patients with cLC at enrollment and HCC development were significantly greater in the groups with higher LSM value (Mantel-Haenszel tests, $P<0.001$, **Figure 2**). In the univariate analysis and subsequent multivariate analysis, together with older age, male gender, heavy alcohol consumption (>80 g/day), lower serum albumin level, and HBeAg positivity, higher LSM values were at a significantly greater risk of HCC development, with a hazard ratio of 3.07 [95% CI, 1.01–9.31; $P=0.047$] when LSM value 8.1–13 kPa, 4.68 [95% CI, 1.40–15.64; $P=0.012$] when LSM value 13.1–18 kPa, 5.55 [95% CI, 1.53–20.04; $P=0.009$] when LSM value 18.1–23 kPa, and 6.60 [95% CI, 1.83–23.84; $P=0.004$] when LSM value >23 kPa, as compared to LSM value ≤8 kPa (**Table 3**).

The cumulative incidence rates of HCC increased significantly in association with elevated LSM value among the 5 stratified groups (log-rank test, $P<0.001$; **Figure 3**). The cumulative incidence rates at 1, 2, and 3 years were 0.17%, 1.12%, and 1.58% in patients with LSM value ≤8 kPa (0.54% per 1 person-year); 1.05%, 2.51%, and 6.28% in patients with 8 kPa< LSM value ≤13 kPa (1.75% per 1 person-year); 2.33%, 5.63%, and 8.77% in patients with 13kPa< LSM value ≤18 kPa (2.94 % per 1 person-year); 0%, 7.86%, and 19.07% in patients with 18 kPa< LSM value ≤23 kPa (7.04% per 1 person-year); 4.48%, 16.8%, and 24.76% in patients with 23 kPa> LSM value (9.80% per 1 person-year).

Table 3. Univariate and Multivariate Cox Proportional Hazard Regression Analysis to Identify Independent Risk Factors for HCC Development

Variables	Univariate	Multivariate	
	P value	Hazard ratio (95% CI)	*P* value
Demographic data			
Age	<0.001	1.06 (1.02-1.09)	<0.001
Male	0.015	3.70 (1.62-8.44)	0.002
Alcohol consumption >80g/day	<0.001	2.30 (1.02-5.20)	0.040
Clinically diagnosed liver cirrhosis	<0.001	-	-
Diabetes mellitus	<0.001	-	-
Previous or ongoing antiviral treatment at enrollment	0.106	-	-
Body mass index		-	-
Underweight			
Normal	0.978		
Overweight	0.981		
Obesity	0.514		
Laboratory data			
Serum albumin	<0.001	0.43 (0.23-0.83)	0.012
Total bilirubin	0.497	-	-
Aspartate aminotransferase	<0.001	-	-
Alanine aminotransferase	0.998	-	-
Prothrombin time	<0.001	-	-
Platelet count	<0.001	-	-
Alpha-fetoprotein	<0.001	-	-
HBeAg positivity	0.019	2.10 (1.02-5.21)	0.012
Detectable HBV-DNA†	0.078	-	-
Liver stiffness measurement			
≤ 8 kPa		1 (reference)	
8.1-13 kPa	<0.001	3.07 (1.01-9.31)	0.047
13.1-18 kPa	<0.001	4.68 (1.40-15.64)	0.012
18.1-23 kPa	<0.001	5.55 (1.53-20.04)	0.009
> 23 kPa	<0.001	6.60 (1.83-23.84)	0.004

Lower limit of detectable HBV-DNA† was 141,000 copies/mL.
HCC, hepatocellular carcinoma; CI, confidence interval; HBeAg, hepatitis B e antigen; kPa, kilopascal.

Figure 2. The proportion of patients with clinically diagnosed liver cirrhosis at enrollment and HCC development based on stratified LSM values.

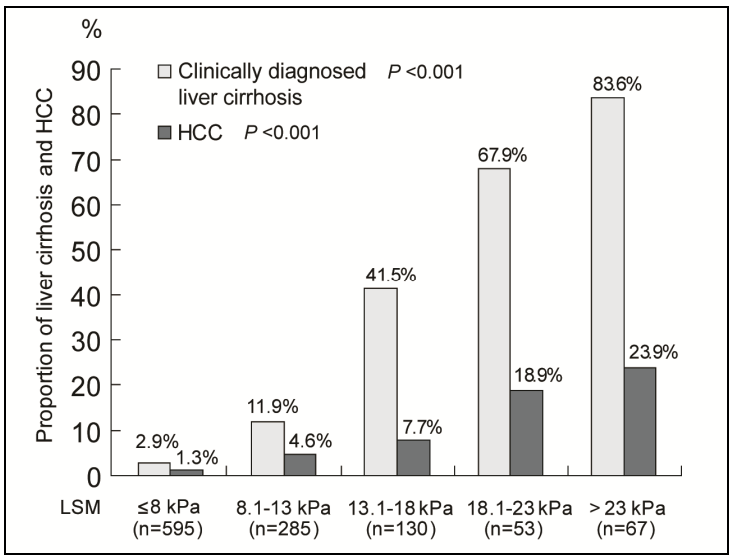
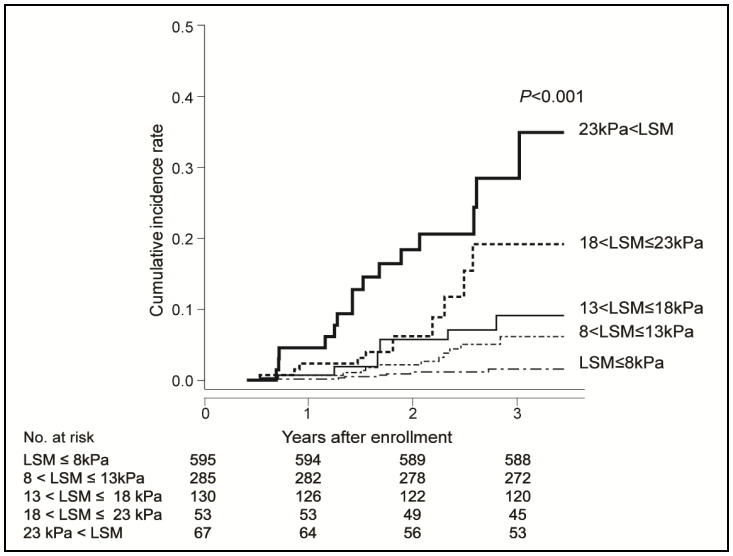


Figure 3. Cumulative incidence rates of HCC based on stratified LSM values (Kaplan-Meier plot).



5. Discordance in the diagnosis of cirrhosis by LSM and clinical criteria

We investigated the discordance that could occur when diagnosing cirrhosis using LSM and clinical criteria, and evaluated any differences in the risk of HCC development. For this sub-analysis, we assessed 1,110 patients without baseline liver histology at enrollment (**Figure 4**).[22] Overall, 874 (78.7%) patients showed LSM value ≤13 kPa and 236 (21.3%) showed LSM value >13 kPa. In patients with LSM value ≤13 kPa, the incidence of HCC estimated by person-years method was not significantly different between patients with cLC (n=45, 5.1%) and those without (n=829, 94.9%) (0.87% *vs*. 0.89% per 1 person-year, *P*=0.518). By contrast, among patients with LSM >13 kPa, HCC developed more frequently when liver cirrhosis was diagnosed according to clinical criteria (n=132, 55.9%) than when it was not (n=104, 44.1%) (5.84% *vs*. 3.26% per 1 person-year, *P*<0.001).

One hundred forty nine (13.4%) patients showed discordance in the diagnosis of cirrhosis when comparing LSM and clinical criteria. The incidence of HCC was higher in 104 patients who showed LSM >13 kPa and no cLC than 45 who showed LSM ≤13 kPa with cLC (3.26% *vs*. 0.87% per 1 person-year) (**Figure 4**).

6. Risk Analysis of HCC Development According to LSM change

After excluding two patients who underwent follow-up LSM after HCC development, 822 patients underwent a second LSM after a median of 18.2 months (range, 11.9–23.0 months) and HCC developed in 26 (3.2%) patients. To estimate the incidence of HCC according to the LSM change, we stratified the patients into four groups as follows: both initial and follow up LSM value ≤13 kPa (Group 1), initial LSM value >13 kPa and follow up LSM value ≤13 kPa (Group 2), initial LSM value ≤13 kPa and follow up LSM value >13 kPa

(Group 3), and both an initial and follow up LSM value >13 kPa (Group 4) (**Figure 5**). In patients with initial LSM value ≤13 kPa (Groups 1 and 3), the patients in Group 3 who had an elevated follow up LSM value had a significantly higher incidence of HCC than those in Group 1 (2.05% [2 of 34 patients] *vs*. 0.44% [7 of 598 patients] per 1 person-year, $P<0.001$), whereas in the patients with an initial LSM value >13 kPa (Groups 2 and 4), patients in Group 2 who had a decreased follow up LSM value had a significantly lower incidence of HCC than those in Group 4 (1.96% [3 of 71 patients] per 1 person-year *vs*. 4.31% [14 of 119 patients] per 1 person-year, $P<0.001$) (**Figure 5**). The chi-square test (Fisher's exact test) revealed that the overall incidence of HCC differed significantly among four groups ($P<0.001$).

7. The incidence of liver-related complications

During the follow up period, liver-related complications developed in 40 patients (3.5%) (variceal bleeding in 27 patients, hepatic encephalopathy in eight, and ascites in five). The stratified LSM was significant only in the univariate analysis ($P=0.045$).

Figure 4. The incidence rates of HCC in patients assessed for liver cirrhosis based on LSM value and clinical criteria at enrollment (n=1,110).

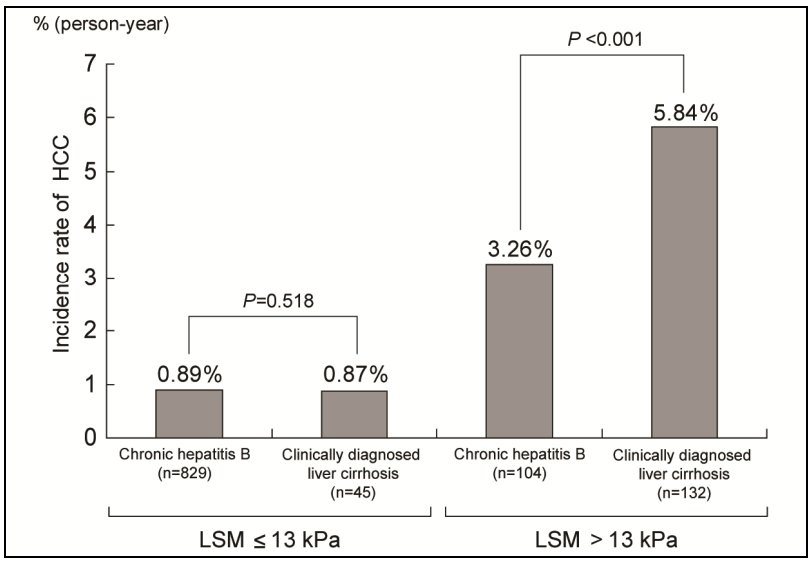
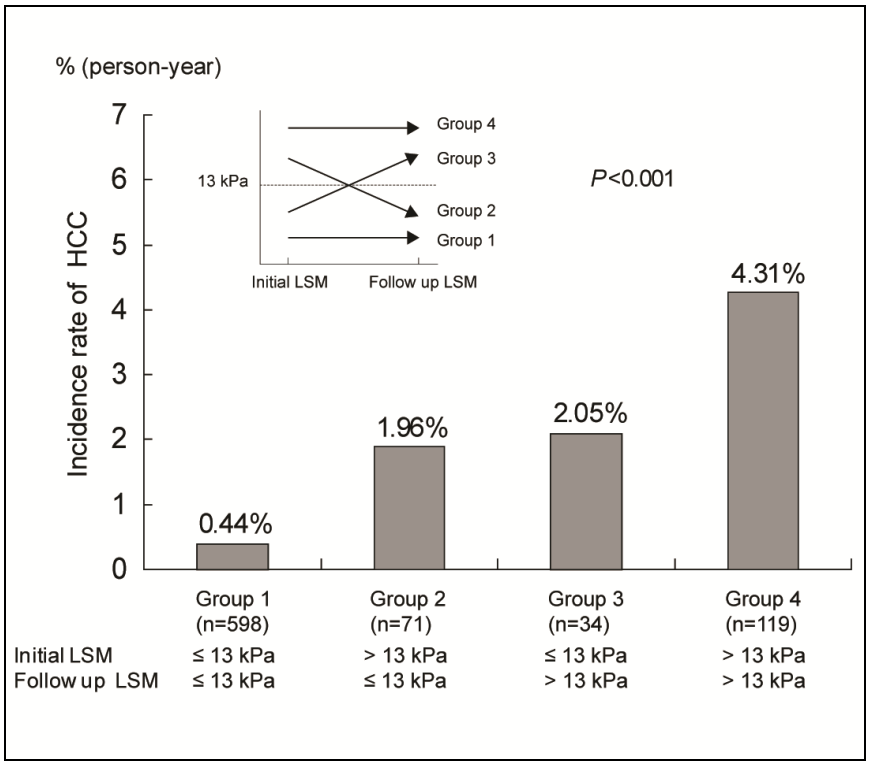


Figure 5. The incidence rates of HCC according to LSM change (n=822).



IV. DISCUSSION

A recent study conducted in Japan proved that LSM predicts HCC development in patients with CHC.[13] With same hypothesis, our study investigated the relationship between LSM and HCC development in patients with CHB, although a significant difference in hepatocarcinogenesis exists between HBV- and HCV-related HCC. HCV-related HCC occurs mostly in the presence of cirrhosis, whereas HBV-related HCC can develop in non-cirrhotic livers, although a strong association between HBV-related HCC and cirrhosis has been found.[25] Despite these different carcinogenetic mechanisms, the usefulness of LSM as a predictor of HCC development in patients with CHB was proved in our study.

The rate of unreliable LSM was much lower in our study (2.2%) than that of a recent European study.[26] Lower BMI and LSM by a single experienced operator might explain this result. Previous studies have reported the annual incidence of HCC in patients with CHB as 0.5–1%, and 2% in those with cirrhosis.[2,4,27] By contrast, the incidence of HCC in our study was 2% per 1 person-year. These results can be explained in several ways. First, because our investigation was based in an urban tertiary teaching hospital, the incidence of HCC might have been overestimated and the proportion of advanced liver disease might be higher than general population of HBsAg bearers. Second, our active surveillance (every 3~6 months with ultrasound and AFP) might be another explanation. Indeed, among the 57 patients with HCC who were detected during surveillance, more than 70% of the patients were operable. On the contrary, the incidence of HCC derived from cirrhosis was lower in our study when compared with previous studies (49.1% *vs.* 70~85%).[25,28] Although the exact reason is not clear, the limited follow-up period of our study might underestimate HCC development from the cirrhotic liver.

Multivariate analysis identified older age, male gender, heavy alcohol

consumption, lower serum albumin, HBeAg positivity, and a high LSM value as independent predictors of HCC development. All these risk factors were similar to those described previously.[29-31] However, in contrast to other reports, our investigations did not identify ALT and detectable HBV DNA as independent predictors of HCC.[4,32] As our exclusion criteria included high risk patients with elevated ALT (>5× the upper limit of normal [ULN]), the overall risk of HCC development in our population may have been lowered. In addition, the initiation of antiviral treatment during the study period may have reduced the incidence of HCC. Although the exclusion of patients with high ALT may result in selection bias, it was necessary to enhance the reliability of LSM.[18,33] In addition, our data did not identify cLC as an independent predictor of HCC development, which is the most important risk factor of HCC development. We believe that the close correlation between cLC and LSM lessened the influence of cLC in the multivariate analysis. This suggests that LSM may be a stronger predictor of HCC than cLC.

When we divided our study population into five groups using the stratified LSM value, the proportion of patients with cLC and HCC development increased significantly in the groups with high LSM values. Furthermore, the stratified LSM value was independently associated with HCC development in our study. These results mean that a correlation between high LSM values and HBV-related HCC development still remained significant, even if HBV-related HCC can develop from a non-cirrhotic background. However, the hazard ratio of HCC development in our patients with CHB was lower than that reported for those with CHC.[13] Indeed, the hazard ratio for HCC development was 45.5 in patients who had CHC with LSM value >25 kPa whereas it was only 6.6 in patients having CHB with LSM value >23 kPa in our study. The hazard ratio for HCC development in our patients may be reduced by HCC cases arising in a non-cirrhotic background. However, as liver cirrhosis defined by LSM value has been identified as a strong independent risk factor for HBV-related HCC

development as in HCV-related HCC,[4,25] we cautiously suggest that LSM can be used as a predictor of HCC development in both HBV and HCV-related CLD. In addition, because 8 kPa has been reported as a cutoff value for significant fibrosis (≥F2)[22,34], our results suggest that patients with significant fibrosis are also at a higher risk of HCC development.

When the incidence of HCC was compared among groups classified using the LSM value and clinical criteria of liver cirrhosis, the incidence of HCC did not differ significantly between patients with LSM ≤13 kPa and cLC and those with LSM ≤13 kPa and without cLC (**Figure 4**). However, the mean LSM values in patients with LSM ≤13 kPa and cLC was significantly higher than that in those with LSM ≤13 kPa and without cLC (9.5 *vs*. 6.9 kPa, $P<0.001$). When compared with patients with LSM >13 kPa and cLC, the proportions of HBeAg positivity (22.2% [n=10] *vs*. 47.0% [n=62]; $P=0.004$) and detectable HBV-DNA (28.9% [n=13] *vs*. 43.2% [n=57]; $P=0.048$) were significantly lower in those with LSM ≤ 13 kPa and cLC. Furthermore, most patients (n=35, 77.8%) had a previous or ongoing use of antiviral agent. Thus, the high proportion of antiviral treatment, lower rate of HBeAg positivity and detectable HBV DNA might have led to completely inactive cirrhosis or resolving fibrosis.[35] This hypothesis might explain the similar incidence of HCC between patients with LSM ≤13 kPa and cLC and those with LSM ≤13 kPa and without cLC.

When we compared two groups with discordance in the diagnosis of cirrhosis by LSM and clinical criteria, the incidence rates of HCC were higher in patients with LSM >13 kPa and no cLC than in those with LSM ≤13 kPa and cLC (**Figure 4**), which might propose the possibility that patients with early compensated liver cirrhosis might have been misstratified as having CHB according to clinical criteria. Finally, when the diagnosis of liver cirrhosis was made by clinical criteria and LSM simultaneously, the incidence of HCC was the highest (5.84% person-year). All these results suggest that LSM might be

more reliable method for diagnosis of compensated liver cirrhosis than clinical criteria, and that LSM can identify the optimal time to recall surveillance program for these high risk patients with compensated liver cirrhosis. Importantly, although the performance of LSM for the prediction of early compensated liver cirrhosis in cross-sectional studies has already been investigated,[20,36] this is the first study to suggest the usefulness of LSM in diagnosis of early compensated liver cirrhosis in a prospective and longitudinal setting by investigating a clinical end-point, defined as the risk of HCC development.

Interestingly, the overall incidence of HCC differed significantly according to the LSM change (**Figure 5**). These results suggest that serial measurements of the LSM value can be used as a dynamic indicator of the risk of HCC development; these findings are supported by previous studies.[37] The incidence of liver-related complications was investigated further. However, the stratified LSM was not significant in the multivariate analysis. However, because the number of HCC development and liver-related complication seems to be still small in our study, confirmative longitudinal observation studies should be followed.

We are aware of the limitations of our study. One major unamendable limitation was the absence of LB data at enrollment. Thus, the exact status of background liver was not informed and we could not provide additional information on the performance of LSM in predicting HCC development in comparison with LB. Another limitation is the method with low sensitivity we used to assess the serum HBV-DNA, which caused difficulties in estimating the association between the serum HBV-DNA level and HCC development and in characterizing our study population completely from inactive carriers to active hepatitis. Third, the relatively short follow-up period can be also one of our limitations. Thus, the role of LSM as a predictor of HCC development in patients with CHB should be confirmed in the future through subsequent studies

with a long-term follow-up period. Lastly, our results can only be applied to a subpopulation of patients with CHB showing limited ALT level (≤5x ULN).[33,38] However, when ALT levels subside after active antiviral treatment in patients with elevated ALT, the reliability of LSM may be restored as indicated in the previous study[38] and LSM may be used as a significant predictor of HCC development.

V. CONCLUSION

In conclusion, this prospective cohort study showed a significant association between LSM and the risk of HCC development in patients with CHB. Therefore, LSM can be used as a noninvasive predictor of HCC development in these patients. Further research is needed to confirm whether surveillance program for HCC in patients with CLD should be adjusted according to LSM.

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ABSTRACT(IN KOREAN)

만성 B형 간염 환자에서 Liver stiffness measurement (FibroScan®)를 통한 간세포암의 발생 위험도 평가

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성 명: 정 규 식

서론: FibroScan®을 이용한 Liver stiffness measurement (LSM)는 만성 C형 간염 환자에서 간섬유화의 정도를 정확히 평가할 뿐 만 아니라, 간세포암 발생의 위험도를 평가하는데도 유용하게 사용 될 수 있다는 것이 최근의 연구에서 보고되었다. 본 연구의 목적은 만성 B형 간염 환자에게서 LSM이 간세포암 발생의 예측인자로 사용될 수 있는지 조사하는 것이다.

재료 및 방법: 2005년 5월부터 2007년 12월까지 총 1130명의 만성 B형 간염 환자가 본 연구에 등록되었다. 등록 후 LSM을 시행하였으며, 이후 주기적으로 간세포암에 대한 검진 검사를 시행하며 추적 관찰하였다.

결과: 전체 환자군의 평균 나이는 50.2 ± 9.9세 였으며, 평균 LSM 수치는 10.3 ± 8.4 kPa 이였다 (남성 767명, 여성 363명). 연구기간 전후로 672명의 환자(59.5%)는 항바이러스 치료를 받았다. 추적 관찰 기간 동안 (중앙값 30.7 개월; 범위 24-50.9 개월) 간세포 암은 57명에서 발생하였으며, 간세포암 발생률은 1인년 당 2.0%였다. 추적 관찰 중 1, 2, 3년의 간세포암의 누적 발생률은 각각 0.80%, 3.26%, 5.98%이었다. 다변량 분석에서, 고령, 남성, 알코올 섭취 80g 이상, 혈중 albumin 농도, HBeAg 양성과 함께 높은 LSM 수치가 통계학적으로 유의한 간세포암 발생의 위험인자로 확인되었다. LSM

수치가 8 kPa 이하인 환자군과 비교하여, 8 kPa 초과하고 13 kPa 이하인 환자군의 경우는 3.07의 상대위험도를 가진 것으로 평가되었으며 (95% 신뢰구간, 1.01–9.31; *P*=0.047), 13 kPa 초과하고 18 kPa 이하인 경우에는 4.68의 상대위험도를 가지는 것으로 (95% 신뢰구간, 1.40–15.64; *P*=0.012), 18 kPa 초과하고 23 kPa 이하인 경우에는 5.55의 상대위험도를 가지는 것으로 (95% 신뢰구간, 1.53–20.04; *P*=0.009), 23 kPa 초과하는 경우에는 6.60 의 상대위험도를 가지는 것으로 평가되었다 (95 % 신뢰구간, 1.83–23.84; *P*=0.004).

결론: 본 연구를 통하여 만성 B형 간염 환자에 있어, LSM이 간세포암의 예측인자로서 사용 될 수 있음을 확인하였다.

핵심되는 말: 만성 B형 간염-Fibroscan-간세포암-Liver stiffness measurement- Transient elastography