Risk Assessment of Hepatocellular Carcinoma in General Population by Liver Stiffness in Combination with Controlled Attenuation Parameter using Transient Elastography: A Cross Sectional Study

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ABSTRACT

Background Hepatocellular carcinoma (HCC) in patients without hepatitis B (HBV) and -C virus (HCV) infection are increasing in Japan. Method for detecting high-risk liver diseases of HCC in general population has still not been established. Liver stiffness measurement (LSM) and Controlled Attenuation Parameter (CAP) using transient elastography (TE; FibroScan System) are useful for detecting liver fibrosis and steatosis. The aim of this study is to clarify TE for risk assessment of HCC in general population.

Methods This cross-sectional study was performed for residents aged ≥ 40 years in an intermountain town in Japan with a population of 3,493. Blood laboratory testing included tumor markers, abdominal ultrasound (AUS), and TE was performed.

Results Among 175 subjects (64 men, 111 women), TE was evaluated and three patients with HCC were detected by AUS. For detecting HCC, the cut-off value of LSM was 5.3 kPa sensitivity 100%, specificity 75%, AUROC 0.88). The combination of LSM and CAP (LSM > 5.3 kPa with any CAP and CAP > 248 dB/m with any LSM) could detect the high-risk liver diseases of HCC (HCC, nonalcoholic fatty liver/steatohepatitis, HBV or HCV related chronic viral hepatitis with alanine transaminase (ALT) > 30 IU/L for men or > 19 IU/L for women or cirrhosis of any cause) with high sensitivity (sensitivity 90%, specificity 55%, positive predictive value 10%, negative predictive value 99%, $P = 0.006$).

Conclusion The combination of LSM and CAP can be useful in detecting high-risk liver diseases of HCC out of general population.

Key words controlled attenuation parameter, liver stiffness measurement, transient elastography, transabdominal ultrasound

Hepatocellular carcinoma (HCC), mainly related to hepatitis C virus (HCV) infection, is a major disease entity with high mortality in Japan.[1] Therefore, screening for hepatitis virus infection had already been performed at periodical health check-ups by a 5-year national project from 2002 to 2006 in Japan.[2, 3] However, only about 60% of these viral positives were admitted to hospitals.[4] The early detection of HCC has not yet been achieved satisfactorily. Recently, the patients without viral infection also have increased in 5 to 20% in Japan,[5] and direct-acting antivirals are estimated to eradicate most of the HCV in the near future.[6] Hence, establishing a new diagnostic method regardless of viral infection is a crucial issue.

A mass-screening program for HCC has never been established in Japan. Although direct screening for all people with abdominal ultrasonography (AUS) is ideal, mass screening is hindered by its slow process (about 15 minutes per person) and cost (around 5000 yen per person).

Liver fibrosis has been reported to be correlated with risk of HCC.[7] Detecting liver steatosis is also important for diagnosing non-alcoholic steatohepatitis (NASH) as a risk of HCC without viral infection.[8] In this regard, liver stiffness measurement (LSM) and Controlled Attenuation Parameter (CAP) using transient elastography (TE; FibroScan System, Echosens SA, Paris, France) can detect non-invasive liver fibrosis and steatosis, respectively.[9–11] They can be measured at the same time. Both LSMs between 7 kPa to 12.5 kPa and > 12.5 kPa are considered as an optimized cutoff value to detect

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Abbreviations: AFP, alpha-fetoprotein; ALT, alanine aminotransferase; AST, aspartate aminotransferase; AUROC, area under the receiver operating characteristic; AUS, abdominal ultrasound; BMI, body mass index; CAP, controlled Attenuation Parameter; DCP, des-γ-carboxy prothrombin; GGT, gamma-glutamyl transferase; HbsAg, hepatitis B surface antigen; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; kPa, kilopascal; LSM, liver stiffness measurement; NAFLD, nonalcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis; ns, not significant; NPV, negative predictive value; PPV, positive predictive value; RAS, the renin–angiotensin system; ROC, receiver operating characteristic; SNS, sympathetic nerve system; TE, transient elastography

significance to sever fibrosis (F2–F3) and cirrhosis (F4) respectively.[9] Recently, several reports have demonstrated that LSM is useful in detection of cirrhosis out of the general population.[12–15] CAP is also reported to be significantly correlated with steatosis found by AUS in the general population.[16] However, to our knowledge, there are no reports evaluating both parameters in HCC screening. The aim of this study is to clarify TE for risk assessment of HCC in general population.

SUBJECTS AND METHODS

Subjects

Firstly, we planned the HCC screening program using tumor markers (alpha-fetoprotein; AFP and des-γ-carboxy prothrombin; DCP) and AUS. Subjects with abnormal findings by AUS and/or elevation in tumor markers (AFP > 10ng /mL, DCP > 40 mAU/mL) were underwent further examinations. The evaluation of sonographic findings was based on the guideline edited by the Japanese society of gastrointestinal cancer screening.[17] In both June 2014 and January 2015, this program was performed for residents of the age of 40 or more in an intermountain town in Japan with a population of 3,493 (population of subjects aged 40 or more was 74.2%). About 40% of the residents aged ≥ 40 of the town were already checked for hepatitis B surface antigen (HBs-Ag) and hepatitis C virus antibody through the national health project,[2, 3] and these data were available. On the same day of the HCC screening program, each applied resident had undergone interviews (alcohol consumption, smoking, comorbidity; diabetes /hypertension/hyperlipidemia), anthropometric measurements (height and weight), blood laboratory tests [aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyl transferase (GGT), AFP, and DCP], AUS, and TE. Drinkers and smokers were defined as alcohol consumption ≥ 20 g/day, and current and former smokers who have more than a 30-pack-per-year history of smoking, respectively.[18, 19] Body mass index (BMI) was calculated as the weight (kg) divided by height (m) squared (kg/m^2). TE was performed by FibroScan 502 equipped with M-probe (Echosens SA, Paris, France). TE was measured through the skin between the rib bones at the level of the right lobe of the liver in the supine position without assistance by AUS. LSM was expressed in kilopascal (kPa) and the median value of 10 measurements was used. At least 10 valid measurements with success rate > 60% and interquartile range/median liver stiffness ratio < 30% were adapted.[20, 21] CAP was also obtained simultaneously. We used the median of individual CAP values expressed in decibels per meter (dB/m). We defined the high-risk liver diseases as HCC, hepatitis B virus (HBV) and hepatitis C virus (HCV) induced chronic viral hepatitis with ALT > 30 IU/L for men or > 19 IU/L for women, nonalcoholic fatty liver disease (NAFLD)/steatohepatitis (NASH) and cirrhosis of any causes.

Ethical Considerations

The study protocol confirmed to the ethical guidelines of the 1975 Declaration of Helsinki as revised in 2000 and was approved by the ethics committee of the Tottori University (No. 2438). Subjects were enrolled after giving their written informed consent.

Statistical analysis

Data are expressed as median (range) or mean ± SD. Statistical analyses for significant differences among the groups were performed using the chi-square test, the Mann-Whitney's *U* test, or the Kruskal-Wallis test. Correlations were calculated using Spearman's rank correlation coefficient. Multivariate analysis was performed using logistic regression model. All statistical analyses were performed using StatFlex (Windows ver 6.0; Artech, Osaka, Japan). Statistical significance was set at $P < 0.05$.

RESULTS

Background of Subjects

We evaluated 181 subjects (65 men, 116 women) in the HCC screening program. In this program, AUS detected three patients (1.7%) with HCC out of 181 subjects in combination with positive tumor markers. The median age was 70 (40–81) years old, the median body mass index (BMI) was 26.7 (15–35) kg/m^2, diabetes in 19 cases (11%), hypertension in 70 cases (38.7%) hyperlipidemia in 61 cases (33.7%) and viral hepatitis in 16 cases (HBV in 2, HCV in 14). Drinkers and smokers were in 36 cases (19.9%) and in 38 cases (21%), respectively (Table 1).

Transient elastography in the HCC screening program

TE could be analyzed in 175 subjects because six subjects were unmeasurable. The average exam duration was 138 ± 106 second, average success rate was 84 ± 23%, the median of LSM was 4.4 (2–27.7) kPa, 7–12.5 kPa in 11 cases (6%), and more than 12.5 kPa in 6 cases (3%) (Table 2). Six (35.3%) of 17 subjects with LSM > 7 kPa had HCV infection. The median LSM values were significantly higher in subjects with BMI ≥ 25 kg/m^2, hepatitis virus infection, habitual smoking, or hypertension than those without these backgrounds (Table 3). On the other hand, the median CAP was 224 (100–389) dB/m (Table 2) and CAP values were significantly higher in subjects with BMI > 25 kg/m^2 and fatty liver detected by

AUS (Table 3). The subjects were divided into quartiles Q1 to Q4, according to CAP values: Q1, < 195; Q2, 195–223; Q3, 224–263; and Q4, ≥ 264 dB/m. Among all the patients, the LSM values were significantly higher in Q4 [4.85 (2.3–16) kPa] compared with Q1 [4.5 (2–13) kPa] and Q3 [3.8 (2.2–20.2) kPa] (P = 0.02) (Fig. 1A). There was no significant correlation between CAP values and LSM values (r = 0.06, P = 0.11) (Fig. 1B). The only one case diagnosed as NASH demonstrated LSM 27.7 kPa and CAP 208 dB/m.

Table 1. Clinical findings in HCC screening program

Subjects		n = 181	
Gender	(male:female)	65:116	
Age	(years)	70	(40–81)
BMI*	(kg/cm^2)	26.7	(15–35)
> 25 kg/cm^2		47	(26%)
HCC		3	(1.7%)
Etiology**			
HBV infection		2	(1.1%)
HCV infection		14	(7.7%)
Alcohol consumption	(≥ 20 g/day)	36	(19.9%)
Smoking	(≥ 30 pack-year)	38	(21%)
Comorbidity			
Diabetes		19	(10.5%)
Hypertension		70	(38.7%)
Hyperlipidemia		61	(33.7%)

Data are expressed as median (range). *Lacked in 2 persons.
**Hepatitis virus infection was checked by hepatitis B surface antigen (HBs-Ag) and HCV antibody.
BMI; body mass index, HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HCV, hepatitis C virus.

LSM in HCC detection

For detecting HCC, the area under the receiver operating characteristic (AUROC) was 0.88. Using the cut-off value of LSM as 5.3 kPa, the sensitivity and specificity were 100% and 75%, respectively (Fig. 2).

Combined parameter for detecting high-risk liver diseases of HCC

Then, we evaluated TE as a tool for detecting high-risk liver diseases of HCC (HCC, HBV and HCV related chronic hepatitis with ALT > 30 IU/L for men or >19 IU/L for women, NAFLD/NASH or cirrhosis of any cause). We have defined a cut-off value of the CAP for the detection of fatty liver by AUS with abnormal ALT (> 30 IU/L for men or > 19 IU/L for women) as 248 dB/m (AUROC 0.77) (Fig. 3), and set the combined parameter as LSM > 5.3 kPa with any CAP and CAP > 248 dB/m with any LSM. The parameter demonstrated high sensi-

Table 2. Results of transient elastography

	n = 181
Success measurement	175 (96.7%)
Exam duration (s)	138 ± 106
Success rates (%)	84 ± 23
LSM (kPa)	4.4 (2–27.7)
7–12.5 kPa	11 (6%)
≥ 12.5 kPa	6 (3%)
CAP (dB/m)	224 (100–389)

Data are expressed as mean ± SD or median (range).
CAP, controlled attenuation parameter; LSM, liver stiffness measurement.

Table 3. Differences of LSM and CAP according to clinical parameters

		n = 175	LSM (kPa)	P	CAP (dB/m)	P
Gender	Male:Female	64:111	4.5 (2–21.8):4.4 (2.2–27.7)	ns	221 (100–389):224 (100–380)	ns
Age	(< 60:≥ 60 yo)	32:143	4.4 (2.2–11.8):4.5 (2–27.7)	ns	230 (133–389):223 (100–380)	ns
BMI	(< 25:≥ 25)	129:44*	4.3 (2–21.8):4.9 (2.3–27.7)	0.010	215 (100–365):256 (100–289)	< 0.001
Hepatitis virus infection	(No:Yes)	160:15	4.4 (2–27.7):6.0 (2.3–21.8)	0.026	226 (100–389):213 (152–314)	ns
Alcohol	(< 20:≥ 20 g/day)	139:36	4.5 (2–27.7):4.35 (2.2–21.8)	ns	224 (100–380):214 (100–389)	ns
Smoking	(< 30:≥ 30 py)	139:36	4.4 (2–27.7):5.0 (2.9–21.8)	0.025	222 (100–389):231 (100–337)	ns
DM	(No:Yes)	156:19	4.4 (2–27.7):4.4 (2.3–11.8)	ns	223 (100–389):247 (100–312)	ns
Hyperlipidemia	(No:Yes)	115:60	4.5 (2–27.7):4.4 (2.2–16)	ns	217(100–389):247(100–331)	ns
Hypertension	(No:Yes)	108:67	4.4 (2–13):4.9 (2.2–27.7)	0.031	222 (100–380):229 (100–389)	ns
Fatty liver by AUS	(No:Yes)	143:32	4.4 (2–27.7):4.9 (2.3–16)	ns	218 (100–365):269 (206–389)	< 0.001

*Lacked in 2 persons.
AUS, abdominal ultrasonography; BMI, body mass index; CAP, controlled attenuation parameter; DM, diabetes mellitus; LSM, liver stiffness measurement; ns, not significant; py, pack-year; yo, year-old.

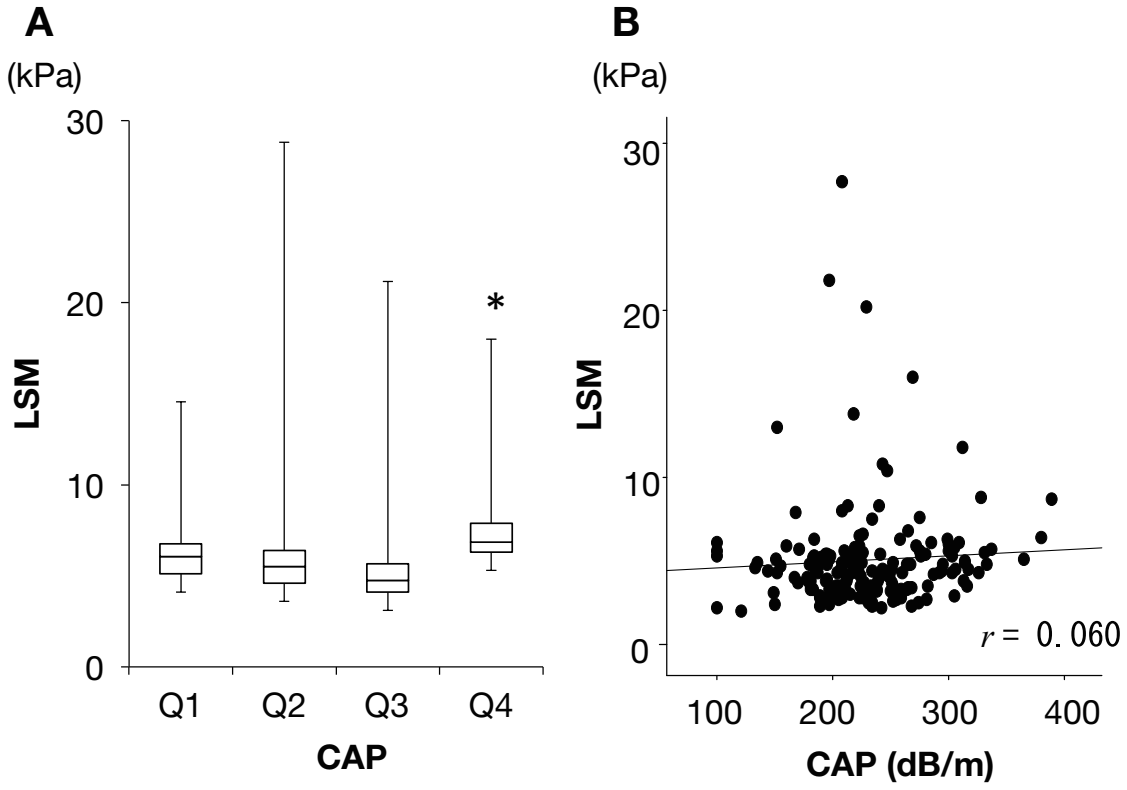

Fig. 1. Relations between LSM and CAP values.
(**A**)The subjects were divided into quartiles Q1 to Q4, according to CAP values: Q1, < 195; Q2, 195–223; Q3, 224–263; and Q4, ≥ 264 dB/m. LSM values were significantly higher in Q4 [4.85 (2.3–16) kPa] compared with Q1 [4.5 (2–13) kPa] and Q3 [3.8 (2.2–20.2) kPa] *P = 0.02 (**B**) There was no significant correlation between CAP values and LSM values (r = 0.06, P = 0.11). CAP, controlled attenuation parameter; LSM, liver stiffness measurement.

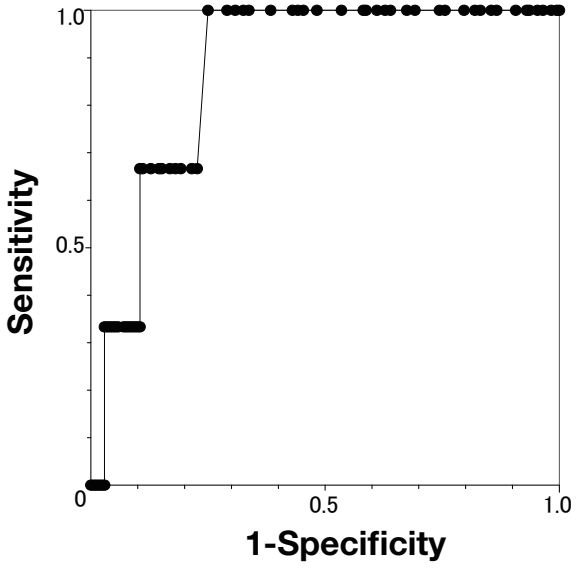

Fig. 2. ROC of the LSM values in detecting HCC.
For detecting HCC, the AUROC was 0.88. Cut-off values of 5.8 kPa had 100% sensitivity and 75% specificity. AUROC, area under the receiver operating characteristic CAP, controlled attenuation parameter; LSM, liver stiffness measurement; ROC, receiver operating characteristic.

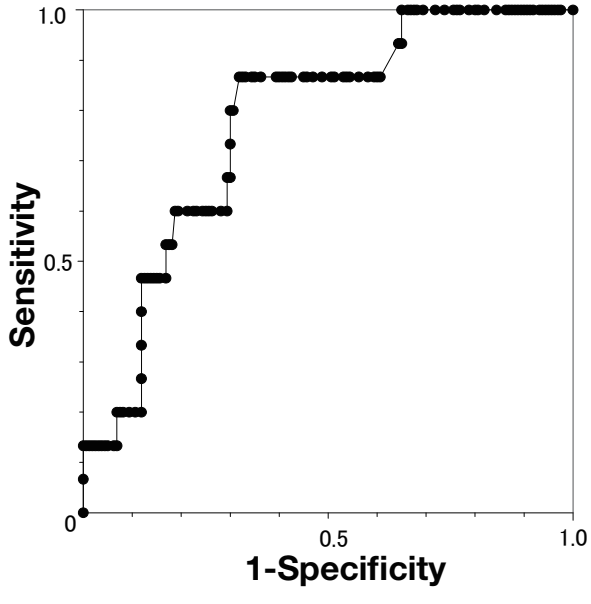

Fig. 3. ROC of the CAP values in detecting fatty liver by AUS with abnormal ALT.
For detecting fatty liver by AUS with abnormal ALT, the AUROC was 0.77. Cut-off values of 248 dB/m had 67% sensitivity and 70% specificity. ALT, alanine aminotransferase; AUS, abdominal ultrasonography; AUROC, area under the receiver operating characteristic; ROC, receiver operating characteristic.

Table 4. Differences between the subjects with and without high-risk liver diseases of HCC

	Non high-risk (n = 164)	High-risk (n = 11)	P
Gender (male:female)	62:102	2:9	ns
Age (years)	70 (40–81)	69 (64–78)	ns
BMI (kg/m^2)	24.6 (15.0–34.3)	21.5 (17.9–28.6)	ns
Hepatitis virus infection	8 (4.9%)	7 (63.6%)	< 0.001
Alcohol consumption (≥ 20 g/day)	34 (20.7%)	2 (18.2%)	ns
Smoking (≥ 30 pack-year)	34 (20.7%)	2 (18.2%)	ns
DM	18 (11%)	1 (9.1%)	ns
AST (IU/L)	23 (14–84)	32 (19–112)	0.001
ALT (IU/L)	17.5 (8–108)	38 (12–93)	0.002
GGT (mg/mL)	21 (9–278)	31 (13–80)	ns
AFP (ng/mL)	2.8 (0.7–9.5)	3.1 (1.2–92)	ns
DCP (mAU/L)	20 (10–281)	27 (10–110)	ns

AFP, alpha-fetoprotein; AST, aspartate aminotransferase; ALT, alanine aminotransferase; BMI, body mass index; CAP, controlled attenuation parameter; DCP, des-γ-carboxy prothrombin; DM, diabetes mellitus; GGT, gamma glutamyl transferase; HCC, hepatocellular carcinoma; LSM, liver stiffness measurement; ns, not significant.

tivity in detecting the high-risk liver diseases [sensitivity 90%, specificity 55%, positive predictive value (PPV) 10%, negative predictive value (NPV) 99%, P = 0.006]. The combined parameter could detect 11 subjects [HCC (n = 3), chronic hepatitis B (n = 1), chronic hepatitis C (n =3), NAFLD (n = 2), NASH (n = 1) and cirrhosis (n = 1)]. The numbers of hepatitis virus infection, AST and ALT levels were significantly higher in the high-risk liver diseases than in the others (Table 4).

DISCUSSION

This study demonstrated the combined parameter of LSM and CAP is a sensitive, non-invasive, quick (about 2 minutes average), and less expensive (around 2000 yen per person) tool in mass screening of high-risk liver diseases.

The detection rate of HCC was low (1.7%) in the present study. The large cohort study for screening HCC using ultrasonography in Japan also demonstrated the incidence of HCC was 0.034% (39/113,992).[22] Taking into consideration the low incidence of HCC in the general population, it is fundamentally difficult to obtain enough sample size for multivariate analysis to predict HCC in such population. Therefore, this preliminary study aimed to evaluate TE for detecting high-risk liver diseases.

Two (66.7%) out of three HCC subjects were HCV positive. The hepatitis virus screening is undoubtedly important for detecting HCC, however, these two patients with known HCV infection had not been indicated any HCCs until this study. Considering their significantly higher LSM, to show 'direct' liver parameters of TE is expected to motivate patients to have ultrasonographic examination.

Liver fibrosis has been reported to be closely related with HCC.[7] Several reports have also demonstrated LSM could indicate the presence of cirrhosis out of general population.[12–15] The selected cutoff level of LSM (5.3 kPa) was relatively lower, however, the optimal cutoff value of LSM is still controversial because of its differences with etiologies.[23] Furthermore, there are no optimal cutoff value for HCC screening.

On the other hand, meta-analysis indicated that the median optimal cut-off value of CAP for stage 1 to 3 steatosis were 232.5 (214–289) dB/m, 255 (233–311) dB/m, and 290 (266–318) dB/m, respectively.[24] In the present study, we set the cutoff value of CAP as 248 dB/m from result of fatty liver diagnosed by AUS with abnormal ALT levels. It covers the stage 1-2 of steatosis.

In this study, the obese subjects had higher LSM and higher CAP than non-obese subjects. Recent report indicated that the LSM values increase according to CAP values, and increase the percentage of false positive.[25] However, in the present study, there was no significant correlation between the two parameters. The LSM values were also significantly higher in smokers and subjects with hypertension. It has been reported that smoking increases hepatic fibrosis when associated with hepatitis virus infection or NAFLD.[26, 27] In the present study, there were only two smokers with hepatitis virus

infection. Except for hepatitis virus infection, there was significant difference in LSM between non-smoker (n = 126) and smoker (n = 34) [4.4 (2–27.7) vs 4.9 (2.9–10.4) kPa, P = 0.028]. CAP values were higher in smokers; however, the rate of fatty liver was not significantly higher in smokers. Moreover, alcohol consumption was higher in smokers; however, no additional influences in LSM were observed. Therefore, there is no convincing data to explain the relation between smoking and liver fibrosis. On the other hand, the subjects with hypertension had higher LSM. Recent reports have also indicated that hypertension is associated with NAFLD as metabolic syndrome.[28] Insulin resistance raises blood pressure through the activation of the sympathetic nerve system (SNS) [29] and the renin–angiotensin system (RAS).[30] The up-regulation of SNS and RAS induces liver fibrosis.[31, 32] Therefore, hypertension can be considered as a part of risk of HCC.

Considering the high sensitivity (90%) and high NPPV (99%), the negativity of the combined parameter would rather effectively exclude the subjects who are supposed not to have high-risk liver disease. In mass screening settings, only the subjects with positive result of combined parameter should be performed further examinations including AUS.

There were several limitations in this study. First, the sample size was small. Therefore, it was difficult to perform multivariate analysis. This is the first preliminary study to clarify the combination of TE parameters for risk assessment of HCC in general population, and it should be validated in a prospective large cohort. Second, six subjects could not be measured by TE; therefore, they were excluded from this study. We used only M probe (3.5MHz), and its unmeasurable rate is reported as 11.6–18.4%, and is associated with BMI > 30 kg/m^2,[23, 33] or age > 50 years.[33] Although these 6 subjects (3%) had significantly higher median BMI than measurable subjects [23.6 vs 26.2 kg/m^2, P = 0.028], there was only one patient with BMI > 30 kg/m^2. On the other hand, all the six subjects were > 50 years old. Nowadays, a new probe (XL probe, 2.5MHz) has become available for obese or older subjects. [23, 33] Further study with XL probe is needed.

In conclusion, the present study demonstrated that the combination of LSM and CAP is a sensitive, non-invasive, quick, and less expensive tool for detecting high-risk liver diseases of HCC out of general population.

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The authors declare no conflict of interest.

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