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ORIGINAL ARTICLE

Repeated liver stiffness measurement compared with paired liver biopsy in patients with non-alcoholic fatty liver disease

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Abstract

Introduction The value of repeated liver stiffness measurement (LSM) in non-alcoholic fatty liver disease (NAFLD) has not been shown before.

Methods A longitudinal study of biopsy-proven NAFLD patients was conducted at the Asian tertiary hospital from November 2012 to January 2017. Patients with paired liver biopsies and LSM were followed prospectively for liver-related and non-liver related complications, and survival.

Results The data for 113 biopsy-proven NAFLD patients (mean age 51.3 ± 10.6 years, male 50%) were analyzed. At baseline, advanced fibrosis based on histology and LSM was observed in 22 and 46%, respectively. Paired liver biopsy and LSM at 1-year interval was available in 71 and 80% of patients, respectively. High-risk cases (defined as patients with advanced fibrosis at baseline who had no fibrosis improvement, and patients who developed advanced fibrosis on repeat assessment) were seen in 23 and 53% of patients, based on paired liver biopsy and LSM, respectively. Type 2 diabetes mellitus was independently associated with high-risk cases. The median follow-up was 37 months with a total follow-up of 328 person-years. High-risk cases based on paired liver biopsy had significantly higher rates of liver-related complications ($p = 0.002$) but no difference in other outcomes. High-risk patients based on paired LSM had a significantly higher rate of liver-related complications ($p = 0.046$), cardiovascular events ($p = 0.025$) and composite outcomes ($p = 0.006$).

Conclusion Repeat LSM can predict liver-related complications, similar to paired liver biopsy, and may be useful in identifying patients who may be at an increased risk of cardiovascular events. Further studies in a larger cohort and with a longer follow-up should be carried out to confirm these observations.

Keywords Liver fibrosis · Cirrhosis · Fibroscan · Liver biopsy · NAFLD

Introduction

Non-alcoholic fatty liver disease (NAFLD) is a condition characterized by the excess accumulation of fat in the liver and is closely related to obesity and the metabolic syndrome. It has emerged as one of the most common causes of chronic liver disease due to the sharp increase in obesity worldwide. A recent meta-analysis has estimated the global prevalence of NAFLD to be 25% [1]. In Malaysia, a study on health check individuals in a private medical facility nearly a decade ago found the prevalence of ultrasonography-diagnosed NAFLD to be 23% [2]. A recent study on health check individuals in a public medical facility found the prevalence of NAFLD based on controlled attenuation parameter (CAP) to be a staggering 57% [3]. Non-alcoholic steatohepatitis (NASH), the more severe form of NAFLD, can lead to liver fibrosis and cirrhosis, with an increased

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risk of developing hepatocellular carcinoma (HCC). NASH has been estimated to affect 3–5% of the general population [4]. In the US, NASH has become the second leading etiology among new liver transplant registrants [5], including that of HCC leading to liver transplantation [6].

A meta-analysis of cohort studies with baseline liver histology identified NAFLD patients with NASH to be at increased risk of liver-related mortality, and the risk was further increased over ten-fold in the presence of advanced fibrosis [7]. There are limited data on the effect of repeated assessment following intervention on the outcomes of NAFLD patients. In addition, there are limited data on liver stiffness measurement (LSM), which has become an increasingly popular tool for the assessment of severity of liver disease in NAFLD patients [8] and the outcomes of these patients. We aimed to prospectively study a well-characterized cohort of biopsy-proven NAFLD patients, focusing on factors associated with advanced fibrosis based on histology and LSM, and the effect of baseline histology and LSM as well as repeated assessments of these on patient outcomes.

Methods

All patients with biopsy-proven NAFLD diagnosed between November 2012 and August 2014 who agreed to participate in this study were included. The diagnosis of NAFLD was made following exclusion of significant alcohol intake ($>$ 21 units per week in men and $>$ 14 units per week in women), use of medications that can cause hepatic steatosis (steroids, amiodarone, methotrexate, tamoxifen, sodium valproate), viral hepatitis B and C infection, and other causes of chronic liver disease, where indicated [9]. Patients were followed every 6–12 months, and demographic, clinical, anthropometric and laboratory data were collected using a standard protocol. Weight and height were measured using standard equipment. Obesity was defined as body mass index (BMI) $\geq$ 25 kg per m^2 [10]. Waist circumference was measured at the midpoint between the lowest margin of the least palpable rib and the top of the iliac crest in the standing position. Central obesity was defined as waist circumference $>$ 90 cm for men and $>$ 80 cm for women [11]. Venous blood was drawn after an overnight fast for complete blood count, blood glucose, glycated hemoglobin (HbA1c), lipid profile and liver profile. Biochemical measurements were performed using standard laboratory procedures.

Liver biopsy and histological assessment

Ultrasonography-guided percutaneous liver biopsy was performed by either one of two experienced operators (W.K.C., S.M.) using an 18-G Temno® II semi-automatic biopsy needle (Cardinal Health, Dublin, OH, USA). Liver biopsy specimens were processed using standard laboratory procedures. Liver biopsy slides were stained with hematoxylin and eosin stain and masson trichrome stain. Liver biopsy slides were examined by an experienced histopathologist (N.R.N.M.) who was blinded to clinical data. NASH was diagnosed based on the presence of steatosis, lobular inflammation, and hepatocyte ballooning. Histopathological findings were reported according to the Non-Alcoholic Steatohepatitis Clinical Research Network (CRN) Scoring System [12]. Representative photos of the scoring from this study can be found in Supplementary File 1 (available online). The NAFLD activity score (NAS) is the sum of scores for hepatic steatosis (0–3), lobular inflammation (0–3) and hepatocyte ballooning (0–2). Fibrosis was staged 0–4 (F0 = no fibrosis, F1 = perisinusoidal or periportal fibrosis, F2 = perisinusoidal and portal/periportal fibrosis, F3 = bridging fibrosis, F4 = cirrhosis). Advanced fibrosis was defined as fibrosis stage $\geq$ F3. The majority of the patients included in this study had a repeat liver biopsy at 1 year as part of a clinical trial, and the histological data were captured for analyses [13].

Transient elastography

Transient elastography was performed after overnight fasting by either one of two experienced operators (W.K.C., S.M.) using Fibroscan 502 Touch with M probe (EchoSens, Paris, France) on the same day of the liver biopsy procedure. Ten valid measurements were obtained for each patient. Adequate pressure of the probe on the skin surface, good layering on TM mode and a straight imaginary line on A mode were ensured for each measurement. An examination was considered successful when valid measurements were $\geq$ 80% and IQR/median for liver stiffness measurement (LSM) was $<$ 30%. Patients with unsuccessful examination were excluded from all analyses on LSM and CAP. Previously reported optimal cut-offs for estimation of the different stages of liver fibrosis were used (5.6 kPa for fibrosis stage $\geq$ F1, 6.65 kPa for fibrosis stage $\geq$ F2, 8 kPa for fibrosis stage $\geq$ F3, and 17 kPa for fibrosis stage F4) [14]. Transient elastography was repeated every 6–12 months. The results of transient elastography at 1 year were used for analyses.

Definition of fibrosis progression and high risk cases

Patients who had an increase of ≥ 1 in fibrosis stage were considered to have fibrosis progression. High-risk cases were defined as patients who had advanced fibrosis at baseline and did not have fibrosis improvement during repeat assessment, and patients who had progressed to advanced fibrosis over the 1-year period. Separate analyses were performed using histology and LSM for case definition.

Cardiovascular events, liver-related complications, malignancy and mortality

Data on cardiovascular events, liver-related complications, malignancy and mortality were captured using a standard protocol up to 31 January 2017. Cardiovascular events included acute myocardial infarction, congestive cardiac failure, need for revascularization and stroke. Liver-related complications included hepatocellular carcinoma, ascites, spontaneous bacterial peritonitis, gastroesophageal varices, gastroesophageal variceal bleeding, hepatic encephalopathy and hepatorenal syndrome. All patients with advanced fibrosis underwent upper endoscopy for variceal screening. Patients who were not found to have esophageal varices were planned for surveillance upper endoscopy after 3 years. The interval for surveillance upper endoscopy will be shortened to a year if a patient develops decompensated cirrhosis. Patients with small esophageal varices were commenced on non-selective beta-blocker and planned for surveillance upper endoscopy in 1 year. Patients with large esophageal varices will undergo endoscopic variceal ligation and be started on non-selective beta-blocker, and have repeat upper endoscopy at 1- to 3-month intervals until complete obliteration of the varices. All patients with advanced fibrosis also had 6-monthly ultrasound examination and measurement of serum alpha-fetoprotein level for hepatocellular carcinoma surveillance. For patients who developed malignancy or died, the type of malignancy and the cause of death was recorded. A composite outcome of cardiovascular events, liver-related complications, malignancy and mortality was also included in the analyses.

Statistical analysis

Data analysis was undertaken using a standard statistical software program (SPSS 16.0; SPSS, Chicago, IL, USA). Continuous variables which were normally distributed were expressed as mean $\pm$ standard deviation and analyzed using the *t* test. Continuous variables which were not normally distributed were expressed as median (interquartile range) and analyzed using the Mann–Whitney test. Categorical variables were expressed as percentages and analyzed using the Chi square test or Fisher's exact test, where appropriate. Multivariate logistic regression models were constructed for patients who had advanced fibrosis based on histology and LSM at baseline, patients who had NASH at baseline, and high-risk patients based on paired liver biopsy and paired LSM. Clinically important and statistically significant variables on univariate analysis were entered into multivariate analyses. Patients were stratified according to the individual histological components, the presence of NASH, the NAS, the grades of steatosis based on CAP, the fibrosis stages based on LSM, the presence of advanced fibrosis based on histology and LSM, and high-risk or non-high-risk cases for outcome analyses. Kaplan–Meier curves were used to illustrate the significant findings of these analyses. Time-to-event analyses were carried out using the log-rank test. For all analyses, a p value of < 0.05 was considered as statistically significant.

Results

Baseline patient characteristics

The study profile is summarized in Fig. 1. Baseline patient characteristics are presented in Table 1. The mean age was 51.3 $\pm$ 10.6 years. There were an equal number of male and female patients. The mean BMI was 29.7 $\pm$ 4.5 kg per m^2, and 86% (97/113) of patients were obese while 95% (107/113) had central obesity. Type 2 diabetes mellitus, hypertension and dyslipidemia were observed in 52% (59/113), 70% (79/113) and 90% (102/113), respectively. Ischemic heart disease was present in 3% (3/113) of patients.

Based on histology, 31% (35/113), 40% (45/113), 7% (8/113), 20% (23/113) and 2% (2/113) of patients had F0, F1, F2, F3 and F4 stages of fibrosis, respectively, at baseline. The characteristics of patients with and without advanced fibrosis based on histology at baseline can be found in Supplementary Table 1 (available online). Multivariate analysis demonstrated the presence of type 2 diabetes mellitus (OR 6.495, 95% CI 1.610–26.196, $p = 0.009$), serum GGT level (OR 1.009, 95% CI 1.002–1.016, $p = 0.011$) and platelet count (OR 0.985, 95% CI 0.975–0.996, $p = 0.008$) to be associated with advanced fibrosis based on histology at baseline (Supplementary Table 2, available online). Seventy-eight percent (88/113) of patients fulfilled the criteria for NASH while the remaining 22% (25/113) did not have NASH. The characteristics of patients with and without NASH at baseline can be found in Supplementary Table 3 (available

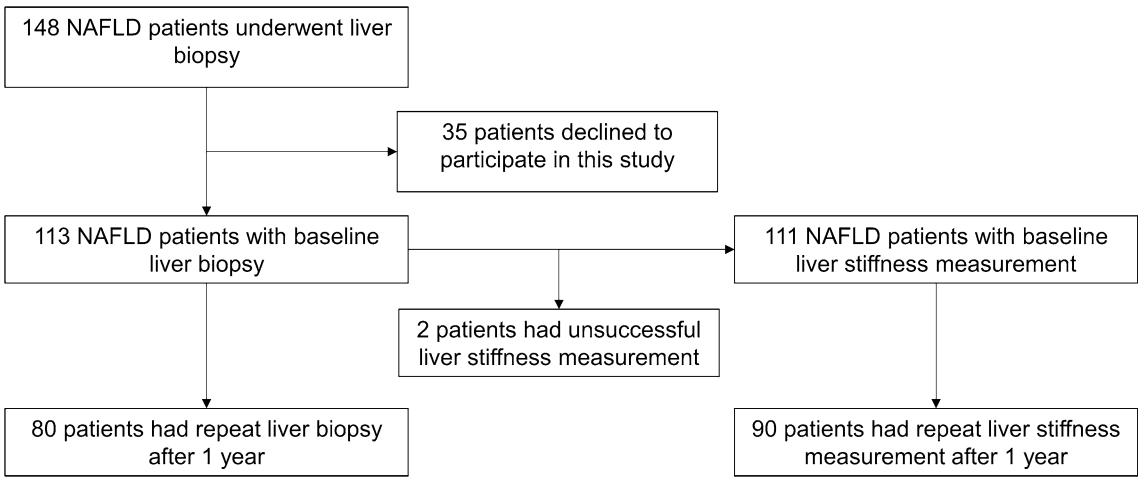


Fig. 1 Study profile

online). Among patients with NASH, 26% (23/88) and 2% (2/88) had F3 and F4 fibrosis, respectively. In contrast, advanced fibrosis was not present in NAFLD patients without NASH. Instead, 76% (19/25) and 24% (6/25) had F0 and F1 fibrosis, respectively. Multivariate analysis demonstrated the presence of central obesity (OR 23.771, 95% CI 1.540–367.049, $p = 0.023$) and type 2 diabetes mellitus (OR 3.929, 95% CI 1.228–12.569, $p = 0.021$) to be associated with NASH (Supplementary Table 4, available online).

Based on LSM, 17% (19/111), 17% (19/111), 20% (22/111), 38% (43/111) and 8% (8/111) of patients had F0, F1, F2, F3 and F4 stages of fibrosis, respectively, at baseline. The characteristics of patients with and without advanced fibrosis based on LSM at baseline can be found in Supplementary Table 5 (available online). Multivariate analysis demonstrated the presence of type 2 diabetes mellitus (OR 4.060, 95% CI 1.627–10.134, $p = 0.003$) and serum ALT level (OR 1.021, 95% CI 1.005–1.038, $p = 0.010$) to be associated with advanced fibrosis based on LSM at baseline (Supplementary Table 6, available online).

Change in fibrosis based on paired liver biopsy and associated factors

Eighty patients (71%) had a repeat liver biopsy at a 1-year interval. Of these patients, 11% (9/80) had fibrosis progression, 65% (52/80) had no change, and 24% (19/80) had fibrosis improvement. In patients who had fibrosis progression, 44% (4/9) developed F4 fibrosis, all of whom had F3 fibrosis at baseline. None of the patients with F0, F1 or F2 stages of fibrosis at baseline developed advanced fibrosis ($\geq$ F3) at 1-year follow-up. The fibrosis stages of these patients at follow-up, stratified according to baseline fibrosis stages, can be found in Supplementary Table 7 (available online).

Nineteen patients were considered to be high-risk cases based on histology. The characteristics of high-risk cases compared with non-high-risk cases based on histology are presented in Table 2. The high-risk cases were older and more likely to have type 2 diabetes mellitus, and had higher FBS, HbA1c and GGT levels and lower platelet count. On multivariate analysis, the presence of type 2 diabetes mellitus (OR 6.484, 95% CI 1.345–31.268, $p = 0.020$) and serum GGT level (OR 1.016, 95% CI 1.004–1.028, $p = 0.007$) were found to be independent factors associated with the high-risk cases (Supplementary Table 8, available online).

Changes in fibrosis based on paired LSM and associated factors

Ninety patients (80%) had paired LSM at 1 year. All patients who had paired liver biopsy had paired LSM except for two patients whose LSM was unsuccessful. Twelve other patients with paired LSM had a baseline liver biopsy but did not undergo a repeat liver biopsy. Of these patients, 19% (17/90) had fibrosis progression, 52% (47/90) had no change, and 29% (26/90) had fibrosis improvement. In patients who had fibrosis progression, four patients developed F4 fibrosis, of which one patient had F2 fibrosis and three patients had F3 fibrosis at baseline. Three patients who had F4 fibrosis at baseline had regression to F3 fibrosis during follow-up. The fibrosis stages of these patients at follow-up, stratified according to baseline fibrosis stages, can be found in Supplementary Table 9 (available online).

Forty-six patients were considered to be high-risk cases based on LSM. The characteristics of high-risk cases compared with non-high-risk cases based on LSM are presented in Table 3. The high-risk cases had a greater BMI and waist circumference, were more likely to have

Table 1 Baseline patient characteristics

Age, years	51.3 ± 10.6
Male, *n* (%)	57 (50)
BMI, kg per m^2	29.7 ± 4.5
Obesity, *n* (%)	97 (86)
Waist circumference, cm	98.5 ± 10.1
Central obesity, *n* (%)	107 (95)
Co-morbidities, *n* (%)	
Type 2 diabetes mellitus	59 (52)
Hypertension	79 (70)
Dyslipidaemia	102 (90)
Ischaemic heart disease	3 (3)
Liver profile	
Albumin, g/L	42 (40–45)
ALT, U/L	70 (48–108)
AST, U/L	42 (29–69)
GGT, U/L	83 (47–135)
Lipid profile	
Triglyceride, mmol/L	1.6 (1.2–2.0)
Total cholesterol, mmol/L	4.7 (4.2–5.5)
HDL, mmol/L	1.1 (1.0–1.3)
LDL, mmol/L	2.8 (2.4–3.5)
Glycemic profile	
Glucose, mmol/L	5.6 (5.0–7.0)
HbA1c, %	6.5 (5.7–7.4)
Platelet count, × 10^9/L	270 (225–300)
Biopsy length, mm	15 (13–17)
Number of portal tracts	8 (6–10)
Steatosis, *n* (%)	
S1	32 (28)
S2	60 (53)
S3	21 (19)
Inflammation, *n* (%)	
0	1 (1)
1	56 (50)
2	51 (45)
3	5 (4)
Ballooning, *n* (%)	
0	24 (21)
1	57 (50)
2	32 (28)
NAS, *n* (%)	
0–2	6 (5)
3–4	51 (45)
≥ 5	56 (50)
Presence of NASH, *n* (%)	
NAFL	25 (22)
NASH	88 (78)
Fibrosis stage based on histology, *n* (%)	
F0	35 (31)
F1	45 (40)
F2	8 (7)
F3	23 (20)
F4	2 (2)
CAP, dB/m	323 (301–346)
LSM, kPa	7.8 (5.9–11.7)
Fibrosis stage based on LSM, *n* (%)	
F0	19 (17)
F1	19 (17)
F2	22 (19)
F3	43 (38)
F4	8 (7)
Missing	2 (2)

BMI body mass index, *ALT* alanine aminotransferase, *AST* aspartate aminotransferase, *GGT* gamma glutamyl transpeptidase, *HDL* high-density lipoprotein, *LDL* low-density lipoprotein, *NAS* NAFLD activity score, *NASH* non-alcoholic steatohepatitis, *NAFL* non-alcoholic fatty liver, *CAP* controlled attenuation parameter, LSM liver stiffness measurement

obesity and type 2 diabetes mellitus, and had higher ALT, AST, GGT, FBS and HbA1c levels. On multivariate analysis, the presence of type 2 diabetes mellitus (OR 7.780, 95% CI 2.430–24.909, $p = 0.001$) and waist circumference (OR 1.087, 95% CI 1.018–1.160, $p = 0.012$) were found to be independent factors associated with the high-risk cases (Supplementary Table 10, available online).

Outcomes during follow-up

The median follow-up was 37 months (range 6–49 months), with a total follow-up of 328 person-years. During follow-up, 4% (4/113) of patients developed liver-related complications and 5% (6/113) developed cardiovascular events. All four liver-related complications were gastro-esophageal varices detected at screening endoscopy. The esophageal varices were small and the patients were commenced on non-selective beta-blocker and had surveillance upper endoscopy every year thereafter. Among the six patients who developed cardiovascular events, two had myocardial infarction, one had revascularization, two had stroke and one required hospitalization for congestive heart failure. The profile of patients who developed cardiovascular event during the study period can be found in Supplementary File 2 (available online). One patient developed gastric cancer, and another died of sepsis.

Patients with advanced fibrosis based on histology at baseline had significantly higher rates of liver-related complications ($p < 0.001$; Fig. 2a). Among the four patients who developed liver-related complications, three

Table 2 Characteristics of high-risk and non-high-risk cases based on paired liver biopsy

	High-risk cases, $n = 19$	Non-high-risk cases, $n = 61$	p
Age, years	56.2 ± 8.1	49.3 ± 11.4	0.017
Male, *n* (%)	8 (42)	28 (46)	0.771
BMI, kg per m^2	30.8 ± 5.4	30.0 ± 4.2	0.502
Obesity, *n* (%)	17 (90)	54 (89)	1.000
Waist circumference, cm	102.1 ± 12.2	98.1 ± 9.9	0.150
Central obesity, *n* (%)	19 (100)	58 (95)	1.000
Co-morbidities, *n* (%)			
Type 2 diabetes mellitus	16 (84)	28 (46)	0.004
Hypertension	16 (84)	44 (72)	0.373
Dyslipidaemia	19 (100)	57 (93)	0.568
Ischaemic heart disease	1 (5)	1 (2)	0.421
Liver profile			
Albumin, g/L	41 (36–44)	42 (39–44)	0.574
ALT, U/L	72 (51–107)	76 (56–124)	0.480
AST, U/L	58 (42–78)	43 (32–72)	0.265
GGT, U/L	138 (77–190)	83 (51–127)	0.003
Lipid profile			
Triglyceride, mmol/L	1.5 (1.1–1.7)	1.6 (1.2–2.0)	0.679
Total cholesterol, mmol/L	4.4 (3.9–5.0)	4.8 (4.2–5.6)	0.118
HDL, mmol/L	1.2 (1.0–1.3)	1.1 (1.0–1.4)	0.764
LDL, mmol/L	2.5 (2.0–3.4)	2.9 (2.4–3.6)	0.081
Glycaemic Profile			
Glucose, mmol/L	6.3 (5.4–8.7)	5.6 (4.9–7.0)	0.026
HbA1c, %	7.2 (6.5–7.8)	6.4 (5.6–7.4)	0.033
Platelet count, $\times 10^9$/L	229 (199–273)	272 (236–308)	0.013
Biopsy length, mm	16 (14–17)	15 (12–17)	0.121
Number of portal tracts	10 (8–11)	8 (6–10)	0.017
Steatosis, *n* (%)			
S1	8 (42)	7 (12)	0.004
S2	10 (53)	35 (57)	
S3	1 (5)	19 (31)	
Inflammation, *n* (%)			
1	5 (26)	21 (34)	0.595
2	12 (63)	37 (61)	
3	2 (11)	3 (5)	
Ballooning, *n* (%)			
0	0 (0)	6 (10)	< 0.001
1	4 (21)	38 (62)	
2	15 (79)	17 (28)	
NAS, *n* (%)			
3–4	6 (32)	20 (33)	0.922
≥ 5	13 (68)	41 (67)	
Presence of NASH, *n* (%)			
NAFL	0 (0)	6 (10)	0.327
NASH	19 (100)	55 (90)	
Fibrosis stage based on histology, *n* (%)			
F0	0 (0)	16 (26)	< 0.001
F1	0 (0)	34 (56)	
F2	0 (0)	7 (11)	
F3	19 (100)	4 (7)	

Table 2 (continued)

	High-risk cases, $n = 19$	Non-high-risk cases, $n = 61$	p
CAP, dB/m	325 (283–370)	326 (306–348)	1.000
LSM, kPa	14.9 (11.6–17.3)	7.8 (5.9–10.5)	< 0.001
Fibrosis stage based on LSM, *n* (%)			
F0	0 (0)	10 (17)	0.002
F1	0 (0)	11 (18)	
F2	1 (5)	12 (20)	
F3	13 (68)	25 (42)	
F4	4 (21)	2 (3)	
Missing	1 (5)	1 (2)	

Patients who had advanced fibrosis at baseline and did not have fibrosis improvement during repeat assessment, and patients who had progressed to advanced fibrosis over the 1-year period were considered to be high-risk cases

Continuous variables which were normally distributed were expressed as mean ± standard deviation and analyzed using *t* tests. Continuous variables which were not normally distributed were expressed as median (interquartile range) and analyzed using the Mann–Whitney test. Categorical variables were expressed as percentages and analyzed using the Chi square test or Fisher's exact test, where appropriate

BMI body mass index, *ALT* alanine aminotransferase, *AST* aspartate aminotransferase, *GGT* gamma glutamyl transpeptidase, *HDL* high-density lipoprotein, *LDL* low-density lipoprotein, *NAS* NAFLD activity score, *NASH* non-alcoholic steatohepatitis, *NAFL* non-alcoholic fatty liver, *CAP* controlled attenuation parameter, *LSM* liver stiffness measurement

had F3 fibrosis, while one had F4 fibrosis at baseline. There was no significant difference in cardiovascular events, malignancy, mortality and composite outcomes between patients with and without advanced fibrosis based on histology at baseline (data not shown). Patients with advanced fibrosis based on LSM at baseline had significantly higher rates of liver-related complications ($p = 0.028$; Fig. 2b). Among the four patients who developed liver-related outcomes, two had LSM consistent with F3 fibrosis, while two had LSM consistent with F4 fibrosis at baseline. There was no significant difference in cardiovascular events, malignancy, mortality and composite outcomes between patients with and without advanced fibrosis based on LSM at baseline (data not shown). The baseline steatosis, lobular inflammation and hepatocyte ballooning grades, the baseline NAS, the presence of NASH, and the baseline CAP did not appear to impact on any of the outcomes (data not shown).

High-risk patients, based on paired liver biopsy, had significantly higher rates of liver-related complications ($p = 0.002$; Fig. 2c), but no difference in other outcomes (data not shown). High-risk patients, based on paired LSM had higher rates of liver-related complications ($p = 0.046$; Fig. 2d). In addition, high-risk patients based on paired LSM also had significantly higher rates of cardiovascular events ($p = 0.025$; Fig. 2e) and composite outcomes ($p = 0.006$; Fig. 2f). There was no difference in malignancy and mortality between high-risk and non-high-risk patients based on paired LSM (data not shown).

Discussion

The use of non-invasive methods to assess the severity of liver disease in NAFLD patients has gained increasing validity. Various non-invasive methods have been developed and validated against the gold standard liver biopsy [3, 15–18]. LSM has one of the better discriminative values for diagnosing fibrosis stage compared with other methods [16, 19]. A meta-analysis of five studies found LSM to be excellent for predicting advanced fibrosis with an AUROC of 0.94 [7]. In the same meta-analysis, the presence of advanced fibrosis on histology was found to be associated with an over 10-fold increase in liver-related mortality among NASH patients. In the current longitudinal study, we found that the presence of advanced fibrosis based on either histology or LSM at baseline was predictive of liver-related complications, indicating that LSM can replace liver biopsy to identify NAFLD patients who are at increased risk of liver-related complications. To the best of our knowledge, there is to date only one other study, by Boursier et al., which has explored baseline LSM and outcomes in NAFLD patients [16]. In that study, NAFLD patients were categorized into four subgroups based on three thresholds of LSM, i.e. 8.8, 12.0 and 38.6 kPa, and the overall survival and survival free from death from liver-related complications were significantly poorer as LSM increased from one group to another. In contrast, our study did not demonstrate a significant difference in overall survival, most likely because of the relatively shorter

Table 3 Characteristics of high-risk and non-high-risk cases based on paired LSM

	High-risk cases, $n = 46$	Non-high-risk cases, $n = 44$	p
Age, years	51.1 ± 11.8	50.6 ± 11.4	0.735
Male, *n* (%)	25 (54)	20 (46)	0.399
BMI, kg per m^2	30.8 ± 4.0	28.1 ± 3.8	< 0.001
Obesity, *n* (%)	44 (96)	34 (77)	0.013
Waist circumference, cm	101.2 ± 9.2	94.5 ± 9.6	< 0.001
Central obesity, *n* (%)	45 (98)	39 (89)	0.107
Co-morbidities, *n* (%)			
Type 2 diabetes mellitus	34 (74)	14 (32)	< 0.001
Hypertension	38 (83)	26 (59)	0.014
Dyslipidaemia	43 (94)	40 (91)	0.711
Ischaemic heart disease	1 (2)	1 (2)	1.000
Liver profile			
Albumin, g/L	43 (40–45)	42 (40–45)	0.894
ALT, U/L	87 (59–134)	64 (45–99)	0.013
AST, U/L	60 (38–77)	36 (29–52)	0.002
GGT, U/L	107 (75–151)	40 (58–110)	0.001
Lipid profile			
Triglyceride, mmol/L	1.7 (1.2–2.0)	1.7 (1.2–2.1)	0.659
Total cholesterol, mmol/L	4.7 (4.0–5.3)	4.8 (4.4–5.7)	0.137
HDL, mmol/L	1.1 (0.9–1.2)	1.2 (1.0–1.4)	0.075
LDL, mmol/L	2.5 (2.3–3.4)	2.9 (2.4–3.4)	0.309
Glycaemic profile			
Glucose, mmol/L	6.3 (5.3–8.0)	5.5 (4.9–6.2)	0.004
HbA1c, %	6.9 (6.1–7.7)	5.8 (5.6–7.2)	0.021
Platelet count, $\times 10^9$/L	253 (203–300)	276.9 ± 61.4	0.237
Biopsy length, mm	16 (13–17)	15 (12–16)	0.120
Number of portal tracts	9 (7–11)	7 (5–9)	0.010
Steatosis, *n* (%)			
S1	10 (22)	9 (20)	0.970
S2	26 (56)	26 (60)	
S3	10 (22)	9 (20)	
Inflammation, *n* (%)			
0	0 (0)	1 (2)	0.003
1	12 (26)	26 (59)	
2	29 (63)	17 (39)	
3	5 (11)	0 (0)	
Ballooning, *n* (%)			
0	2 (4)	13 (30)	< 0.001
1	22 (48)	24 (54)	
2	22 (48)	7 (16)	
NAS, *n* (%)			
0–2	0 (0)	3 (7)	0.011
3–4	13 (28)	22 (50)	
≥ 5	33 (72)	19 (43)	

Table 3 (continued)

	High-risk cases, $n = 46$	Non-high-risk cases, $n = 44$	p
Presence of NASH, *n* (%)			
NAFL	2 (4)	14 (32)	0.001
NASH	44 (96)	30 (68)	
Fibrosis stage based on histology, *n* (%)			
F0	6 (13)	18 (41)	< 0.001
F1	14 (31)	24 (55)	
F2	6 (13)	1 (2)	
F3	19 (41)	1 (2)	
F4	1 (2)	0 (0)	
CAP, dB/m	326 (311–361)	318 (297–342)	0.052
LSM, kPa	11.8 (8.6–15.6)	6.4 (5.3–7.6)	< 0.001
Fibrosis stage based on LSM, *n* (%)			
F0	1 (2)	13 (30)	< 0.001
F1	4 (9)	11 (25)	
F2	5 (11)	13 (30)	
F3	30 (65)	7 (15)	
F4	6 (13)	0 (0)	

Patients who had advanced fibrosis at baseline and did not have fibrosis improvement during repeat assessment, and patients who had progressed to advanced fibrosis over the 1-year period were considered to be high-risk cases

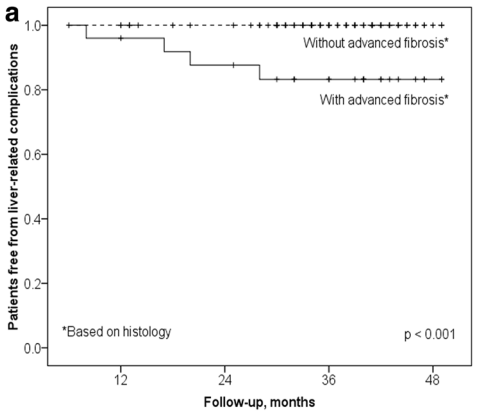
Continuous variables which were normally distributed were expressed as mean ± standard deviation and analyzed using the *t* test. Continuous variables which were not normally distributed were expressed as median (interquartile range) and analyzed using Mthe ann–Whitney test. Categorical variables were expressed as percentages and analyzed using the Chi square test or Fisher's exact test, where appropriate

BMI body mass index; *ALT* alanine aminotransferase; *AST* aspartate aminotransferase; *GGT* gamma glutamyl transpeptidase; *HDL* high-density lipoprotein; *LDL* low-density lipoprotein; *NAS* NAFLD activity score; *NASH* non-alcoholic steatohepatitis; *NAFL* non-alcoholic fatty liver; *CAP* controlled attenuation parameter; LSM liver stiffness measurement

follow-up period and the smaller number of deaths. None of our patients had succumbed to liver-related complications at the time of evaluation. We also found that histological markers such as baseline steatosis, lobular inflammation and hepatocyte ballooning grades, the baseline NAS, the presence of NASH, and the baseline CAP did not have impact on any of the outcomes. These findings are consistent with that from another longitudinal study of 229 biopsy-proven NAFLD patients, in which advanced fibrosis stage, not the NAS, was found to be predictive of overall and disease-specific mortality [20].

To date, there has not been a universally accepted cut-off for the diagnosis of advanced fibrosis in NAFLD patients. We decided to use the cut-offs by Yoneda et al., because those were the cut-offs that have been used since the Fibroscan became available at our center in 2009 until today [14]. The 8-kPa cut-off used for the diagnosis of advanced fibrosis in our study falls within the range of accepted cut-offs for advanced fibrosis. For example, in a highly-cited study on 246 biopsy-proven NAFLD patients, the cut-off for advanced fibrosis to yield above 90% sensitivity, maximum sum of sensitivity and specificity, and above 90% specificity was 7.9, 8.7 and 9.6 kPa, respectively [8]. Using this as a comparison, the 8-kPa cut-off that we have used in our study would translate to a higher sensitivity for the diagnosis of advanced fibrosis at the expense of specificity. This is also the reason for a much larger proportion of patients diagnosed to have advanced fibrosis based on LSM compared with histology (e.g., 46 vs. 22% at baseline).

Our study demonstrated that the pre-defined high-risk group identified based on paired LSM had significantly higher cardiovascular events compared to the non-high-risk group. This, in addition to the significantly higher liver-related complications, contributed to the significantly higher composite outcome seen in this high-risk group. The significantly higher cardiovascular events and composite outcomes were not observed when the definition of high-risk group was based on histology. As LSM may be affected by the skin–capsular distance, it is possible that increments in LSM also partly reflected worsening of adiposity and metabolic profile of these high-risk patients, hence the association with cardiovascular events. In a study on 169 mostly overweight or obese NAFLD patients by

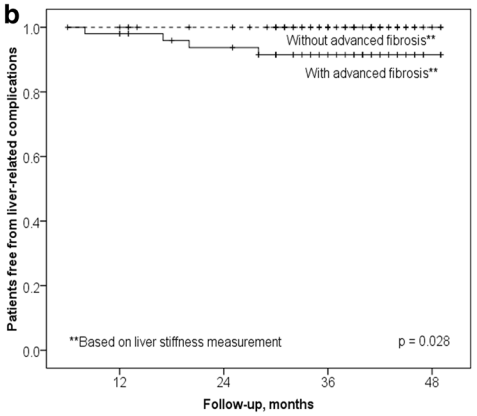


Without advanced fibrosis (based on histology)

88	87	78	50	4

With advanced fibrosis (based on histology)

25	24	21	16	2

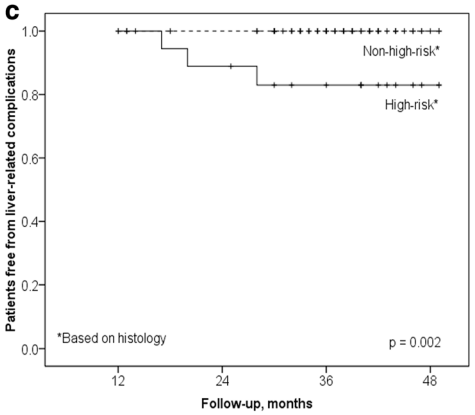


Without advanced fibrosis (based on liver stiffness measurement)

60	59	54	37	3

With advanced fibrosis (based on liver stiffness measurement)

51	50	43	27	3

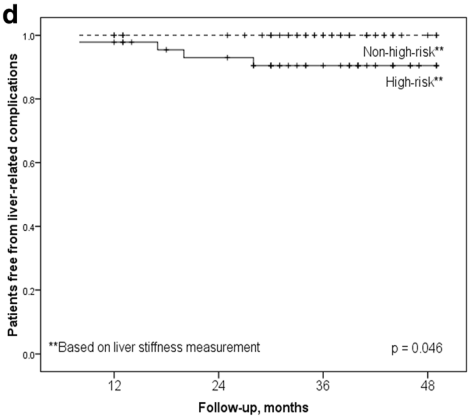


Non-high risk (based on histology)

61	61	54	36	4

High-risk (based on histology)

19	19	16	12	2

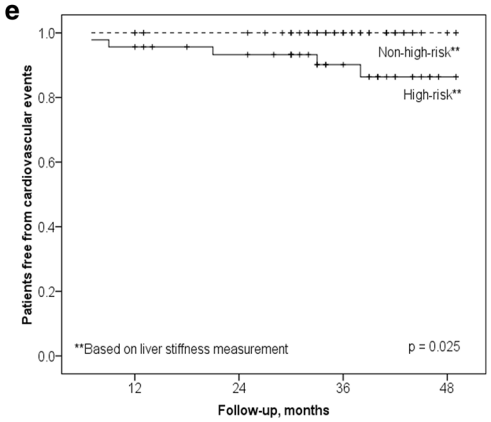


Non-high risk (based on liver stiffness measurement)

44	44	40	25	3

High-risk (based on liver stiffness measurement)

46	45	38	23	3

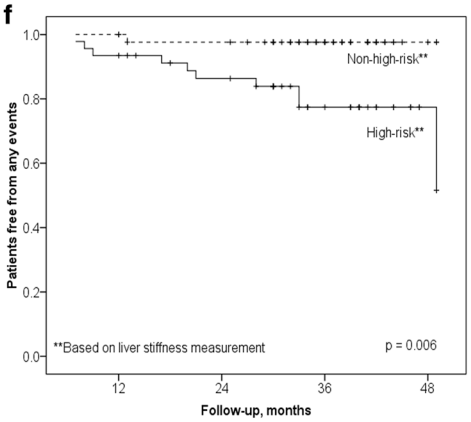


Non-high-risk (based on liver stiffness measurement)

44	44	40	25	3

High-risk (based on liver stiffness measurement)

46	44	39	25	3



Non-high-risk (based on liver stiffness measurement)

44	44	40	25	3

High-risk (based on liver stiffness measurement)

46	43	36	21	3

◀**Fig. 2** Kaplan–Meier curves showing **a** patients free from liver-related complications stratified according to presence of advanced fibrosis based on histology at baseline, **b** patients free from liver-related complications stratified according to presence of advanced fibrosis based on LSM at baseline, **c** patients free from liver-related complications stratified according to pre-defined high-risk criteria based on paired histology, **d** patients free from liver-related complications stratified according to pre-defined high-risk criteria based on paired LSM, **e** patients free from cardiovascular events stratified according to pre-defined high-risk criteria based on paired LSM, and **f** patients free from any events stratified according to pre-defined high-risk criteria based on paired LSM

Petta et al., BMI (and conceivably skin–capsular distance) was clearly demonstrated to affect LSM [21]. For example, the false positive rate for diagnosis of advanced fibrosis increased from 11% in those with lower BMI to 38% among those with higher BMI. Our study was not aimed to determine whether the association of the high-risk group with cardiovascular events was independent of other factors, which requires further studies with a larger number of subjects for a longer period of time and with a larger number of cardiovascular events.

There is a paucity of published data with paired liver biopsy in Asian NAFLD patients. We previously reported on 35 patients with biopsy-proven NAFLD who did not receive any specific intervention and who had a repeat liver biopsy after a mean interval of 6 years [22]. In that study, fibrosis worsened in 51% (18/35) of patients and remained unchanged in 49% (17/35). None of the patients had fibrosis improvement. We could not identify any factors associated with worsened fibrosis in that study, most likely because of the small number of subjects. Moreover, without any patients with fibrosis improvement, the magnitude of effect of any factors for worsened fibrosis may have been attenuated. In the current study, we found the presence of type 2 diabetes mellitus to be independent factors associated with a pre-defined high-risk criteria based on paired liver biopsy as well as paired LSM. This too is not dissimilar to a study on 108 NAFLD patients with paired liver biopsies, which demonstrated a higher prevalence of type 2 diabetes mellitus in patients who had worsened fibrosis compared to those who did not (84 vs. 51%, $p < 0.001$) [23]. In fact, a recent study on a large cohort of patients with type 2 diabetes mellitus found increased CAP (consistent with significant hepatic steatosis) and increased LSM (consistent with advanced fibrosis) in 73 and 18%, respectively, supporting the screening of patients with type 2 diabetes mellitus for NAFLD and advanced fibrosis [24].

The main strength of our study is the availability of paired liver biopsy as well as paired LSM at a 1-year interval that has enabled us to identify a subgroup of high-risk patients with NAFLD. Furthermore, the availability of longitudinal data has enabled us to explore the outcomes of these patients. One of the limitations of this study is the small number of events, which has limited our ability to perform multivariate analyses to look for independent factors associated with the various outcomes. Nevertheless, the study did provide some useful insights into the impact of histology and LSM, both at baseline and in a paired manner, with the various outcomes. Moreover, this is considered a relatively large cohort of NAFLD patients with paired liver biopsy and LSM for a single-center prospective study. Future longitudinal studies on NAFLD patients should take into account the low event rates, and should aim to enroll a larger number of subjects and to follow them for a longer period of time. As this study included biopsy-proven NAFLD patients from a speciality clinic in a tertiary hospital, and included a large proportion of NASH patients with the metabolic syndrome, the findings may not be applicable to NAFLD patients in the general population or in a primary care setting. Studies using liver histology may also be limited by sampling variability. While the mean length and number of portal tracts of the liver biopsy specimen of our study population fell short of the recommended standard, none of the specimens were deemed to be inadequate for evaluation.

In conclusion, our study found the presence of type 2 diabetes mellitus to be an independent factor associated with advanced fibrosis and high-risk cases in NAFLD patients. LSM is as good as liver biopsy in identifying NAFLD patients with increased risk of liver-related complications. In addition, repeating LSM at a 1-year interval may be useful to identify patients who may be at an increased risk of cardiovascular events. Further studies of a larger cohort and with a longer follow-up should be carried out to confirm these observations.

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Compliance with ethical standards

Conflict of interest All authors declare that they have no competing interest.

Ethical approval This prospective study was approved by the University of Malaya Medical Centre (UMMC) Medical Ethics Committee (MEC ID no.: 20168124134).

Informed consent All patients who participated in this study provided written informed consent.

References

1. Younossi ZM, Koenig AB, Abdelatif D, Fazel Y, Henry L, Wymer M. Global epidemiology of nonalcoholic fatty liver disease-meta-analytic assessment of prevalence, incidence, and outcomes. Hepatology. 2016;64:73–84.
2. Goh SC, Ho EL, Goh KL. Prevalence and risk factors of non-alcoholic fatty liver disease in a multiracial suburban Asian population in Malaysia. Hepatol Int. 2013;7:548–54.
3. Tan EC, Tai SM, Chan WK, et al. A study of non-alcoholic fatty liver disease using transient elastography and carotid intima media thickness using ultrasonography in a middle-aged Malaysian population. J Gastroenterol Hepatol. 2016;31(Suppl. 3):7–441.
4. Vernon G, Baranova A, Younossi ZM. Systematic review: the epidemiology and natural history of non-alcoholic fatty liver disease and non-alcoholic steatohepatitis in adults. Aliment Pharmacol Ther. 2011;34:274–85.
5. Wong RJ, Aguilar M, Cheung R, Perumpail RB, Harrison SA, Younossi ZM, Ahmed A. Nonalcoholic steatohepatitis is the second leading etiology of liver disease among adults awaiting liver transplantation in the United States. Gastroenterology. 2015;148:547–55.
6. Wong RJ, Cheung R, Ahmed A. Nonalcoholic steatohepatitis is the most rapidly growing indication for liver transplantation in patients with hepatocellular carcinoma in the US. Hepatology. 2014;59:2188–95.
7. Musso G, Gambino R, Cassader M, Pagano G. Meta-analysis: natural history of non-alcoholic fatty liver disease (NAFLD) and diagnostic accuracy of non-invasive tests for liver disease severity. Ann Med. 2011;43:617–49.
8. Wong VW, Vergniol J, Wong GL, Foucher J, Chan HL, Le Bail B, Choi PC, et al. Diagnosis of fibrosis and cirrhosis using liver stiffness measurement in nonalcoholic fatty liver disease. Hepatology. 2010;51:454–62.
9. Chalasani N, Younossi Z, Lavine JE, Diehl AM, Brunt EM, Cusi K, Charlton M, et al. The diagnosis and management of non-alcoholic fatty liver disease: practice Guideline by the American Association for the Study of Liver Diseases, American College of Gastroenterology, and the American Gastroenterological Association. Hepatology. 2012;55:2005–23.
10. Anuurad E, Shiwaku K, Nogi A, Kitajima K, Enkhmaa B, Shimono K, Yamane Y. The new BMI criteria for asians by the regional office for the western pacific region of WHO are suitable for screening of overweight to prevent metabolic syndrome in elder Japanese workers. J Occup Health. 2003;45:335–43.
11. Alberti KG, Zimmet P, Shaw J. The metabolic syndrome–a new worldwide definition. Lancet. 2005;366:1059–62.
12. Kleiner DE, Brunt EM, Van Natta M, Behling C, Contos MJ, Cummings OW, Ferrell LD, et al. Design and validation of a histological scoring system for nonalcoholic fatty liver disease. Hepatology. 2005;41:1313–21.
13. Wah Kheong C, Nik Mustapha NR, Mahadeva S. A randomized trial of Silymarin for the treatment of nonalcoholic steatohepatitis. Clin Gastroenterol Hepatol. 2017;15(1940–1949):e1948.
14. Yoneda M, Fujita K, Inamori M, Nakajima A, Yoneda M, Tamano M, Hiraishi H. Transient elastography in patients with non-alcoholic fatty liver disease (NAFLD). Gut. 2007;56:1330–1.
15. Karlas T, Petroff D, Sasso M, Fan JG, Mi YQ, de Ledinghen V, Kumar M, et al. Individual patient data meta-analysis of controlled attenuation parameter (CAP) technology for assessing steatosis. J Hepatol. 2017;66:1022–30.
16. Boursier J, Vergniol J, Guillet A, Hiriart JB, Lannes A, Le Bail B, Michalak S, et al. Diagnostic accuracy and prognostic significance of blood fibrosis tests and liver stiffness measurement by FibroScan in non-alcoholic fatty liver disease. J Hepatol. 2016;65:570–8.
17. Friedrich-Rust M, Ong MF, Martens S, Sarrazin C, Bojunga J, Zeuzem S, Herrmann E. Performance of transient elastography for the staging of liver fibrosis: a meta-analysis. Gastroenterology. 2008;134:960–74.
18. Unalp-Arida A, Ruhl CE. Noninvasive fatty liver markers predict liver disease mortality in the US population. Hepatology. 2016;63:1170–83.
19. Seki K, Shima T, Oya H, Mitsumoto Y, Mizuno M, Okanoue T. Assessment of transient elastography in Japanese patients with non-alcoholic fatty liver disease. Hepatol Res. 2017;47:882–9.
20. Ekstedt M, Hagstrom H, Nasr P, Fredrikson M, Stal P, Kechagias S, Hultcrantz R. Fibrosis stage is the strongest predictor for disease-specific mortality in NAFLD after up to 33 years of follow-up. Hepatology. 2015;61:1547–54.
21. Petta S, Di Marco V, Camma C, Butera G, Cabibi D, Craxi A. Reliability of liver stiffness measurement in non-alcoholic fatty liver disease: the effects of body mass index. Aliment Pharmacol Ther. 2011;33:1350–60.
22. Chan WK, Ida NH, Cheah PL, Goh KL. Progression of liver disease in non-alcoholic fatty liver disease: a prospective clinicopathological follow-up study. J Dig Dis. 2014;15:545–52.
23. McPherson S, Hardy T, Henderson E, Burt AD, Day CP, Anstee QM. Evidence of NAFLD progression from steatosis to fibrosing-steatohepatitis using paired biopsies: implications for prognosis and clinical management. J Hepatol. 2015;62:1148–55.
24. Kwok R, Choi KC, Wong GL, Zhang Y, Chan HL, Luk AO, Shu SS, et al. Screening diabetic patients for non-alcoholic fatty liver disease with controlled attenuation parameter and liver stiffness measurements: a prospective cohort study. Gut. 2016;65:1359–68.