Non-invasive risk-based surveillance of hepatocellular carcinoma in patients with metabolic dysfunction-associated steatotic liver disease

Short title: HCC surveillance in MASLD

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This study involves human participants and the protocol of this study underwent approval by the Joint Chinese University of Hong Kong-New Territories East Cluster Clinical Research Ethics Committee (Reference number: 2022.255) and the institutional review boards of all participating centres. Adherence to the Declaration of Helsinki principles was undertaken and the need for informed written consent was waived due to its retrospective nature.

Data sharing statement

Data are available upon reasonable request to corresponding authors.

Author contributions:

VW-SW and TC-FY designed the study. JC-TL, BY, TC-FY, HWL, HL, ET, SP, EB, MY, M-HZ, HH, JB, JLC, GB-BG, W-KC, RG-D, AJS, VdL, PNN, J-GF, GL-HW, GP, AA, AN, W-YL, YS, MdS-L, EL, KKJT, CL-R, AA, SM, CMC, MR-G, SUK and VW-SW collected data in this study. ET, SP, EB, M-HZ, HH, JB, JLC, GB-BG, W-KC, AJS, VdL, PNN, MR-G, SUK and VW-SW supervised the project. JC-TL, BY, TC-FY, and VW-SW were responsible for data analysis and data interpretation, and drafted the manuscript. BY and TC-FY prepared the figures. All authors provided review and editing of the manuscript, and approved the final version of the manuscript. TC-FY, VW-SW, and SUK are the guarantors of the study.

Potential conflict-of-interest statements:

JC-TL served as a speaker for Gilead Sciences and Abbott, and an advisory board committee for Gilead Sciences and Boehringer Ingelheim. ET served as a consultant for Pfizer, NovoNordisk, Boehringer, and Siemens Healthineers; and a speaker for NovoNordisk, Echosens, and Dr Falk. SP served as a speaker or advisor for AbbVie, Echosens, MSD, Novo Nordisk, Pfizer, and Resalis. EB served as a consultant for Boehringer, MSD, Novo Nordisk, and Pfizer; and a speaker for MSD, Novo Nordisk, and Madrigal. She received research grants from Gilead Sciences. MY received research grant from Gilead Sciences and speaker for KOWA. AN received research grants from Mochida Pharmaceutical, Astellas Pharma, ASKA pharmaceutical, Biofermin Pharmaceutical and EA pharma; a speaker for Mochida Pharmaceutical, Kowa, Biofermin Pharmaceutical, MSD, Boehringer, Novo Nordisk, GlaxoSmithkline, EA pharma. HH served as a consultant for AstraZeneca; and a hepatic events adjudication committee member for KOWA and GW Pharama. His institution has received research funding from AstraZeneca, Echosens, Gilead Sciences, Intercept, MSD, and Pfizer. JB served as a consultant for AstraZeneca, Echosens, Intercept, and Siemens; a speaker for AbbVie, Gilead Sciences, Intercept, and Siemens; and an advisory board member for Bristol-Myers-Squibb, Intercept, Pfizer, MSD, and Novo Nordisk. His institution has received research funding from Diafir, Echosens, Intercept, Inventiva, and Siemens. JLC served as a consultant and speaker for

Echosens, Gilead Sciences, and AbbVie. GB-BG served as a consultant for Roche and Ionis Pharmaceuticals; and a speaker for Echosens, Viatris, Abbott and Novo Nordisk. WKC served as a consultant for Abbott, Roche, AbbVie, Novo Nordisk, and Boehringer Ingelheim; and a speaker for Abbott, Novo Nordisk, Echosens, Hisky Medical, and Viatris. AJS served as a consultant for 89Bio, Akero, Allergan, Alnylam Pharmaceuticals, Amgen Inc, AstraZeneca, Boehringer Ingelheim, Bristol-Myers Squibb, Genentech, Gilead Sciences, Histoindex, Intercept Pharmaceuticals, Inventiva, Madrigal, Merck, Novartis, Novo Nordisk, Pfizer, Poxel, Salix Pharmaceuticals, Siemens, Sun Pharmaceutical Industries Inc, Terns, and Valeant Pharmaceuticals; and a data safety monitoring board or advisory board member for Bard Peripheral Vascular Inc, NGM Biopharmaceuticals, and Sequana. He has received research funding from Albireo, Allergan, Echosens, Eli Lilly, Gilead Sciences, Intercept Pharmaceuticals, Mallinckrodt LLC, Merck, Novo Nordisk, Perspectum, Pfizer, Salix Pharmaceuticals, and Zydus; and holds the stocks of Durect, Exhalenz, Gen t, and Tiziana. MR-G served as a consultant for Siemens; and a speaker for Siemens and Echosens. He has received research funding from Siemens, Echosens, and Novo Nordisk. PN served as a consultant for Novo Nordisk, Boehringer Ingelheim, Gilead Sciences, Intercept, Poxel Pharmaceuticals, Pfizer, BMS, Eli Lilly, Madrigal, and GSK; and a speaker for Novo Nordisk and AiCME. He has received research funding from Novo Nordisk. LC served as a consultant for Boston pharmaceutical, Echosens, Gilead, GSK, Madrigal, MSD, Novo Nordisk, Pfizer, Sagimet and Siemens Healthineers and as speaker for Echosens, Gilead, Inventiva, Madrigal and Novo Nordisk. CF-P is an employee of Echosens. GL-HW served as a consultant for AstraZeneca, Gilead Sciences, GlaxoSmithKline and Janssen; and a speaker for Abbott, Abbvie, Bristol-Myers Squibb, Echosens, Furui, Gilead Sciences, GlaxoSmithKline and Roche. She has received research funding from Gilead Sciences. MS-WC is an employee of Echosens. VW-SW served as a consultant or advisory board member for AbbVie, Boehringer Ingelheim, Echosens, Gilead Sciences, Intercept, Inventiva, Novo Nordisk, Pfizer, Sagimet Biosciences, TARGET PharmaSolutions, and Visirna; and a speaker for Abbott, AbbVie, Gilead Sciences, Novo Nordisk, and Unilab. He has received a research grant from Gilead Sciences, and is a co-founder of Illuminatio Medical Technology. TC-FY has served as an advisory committee member and a speaker for Gilead Sciences. The other authors declare that they have no competing interests.

ABSTRACT

Background: Metabolic dysfunction-associated steatotic liver disease (MASLD) affects over 30% of the general population and is the fastest-growing cause of hepatocellular carcinoma (HCC). Current guidelines recommend HCC surveillance in patients with cirrhosis when annual HCC incidence exceeds 1% without specifying the role of non-invasive tests in patient selection.

Objective: To define non-invasive test thresholds to select patients with MASLD for HCC surveillance.

Design: A multicentre longitudinal study of adults with MASLD from 16 tertiary centres in the US, Europe and Asia between February 2004 and January 2023. Primary outcome was incident HCC.

Results: 12,950 patients had FIB-4 and LSM (mean age 51.7 years; 41.1% male). At a median follow-up of 47.7 (IQR, 23.3-72.3) months, 109 (0.8%) developed HCC. FIB-4 was below the low cut-off (<1.3 if aged <65 years, and <2.0 if aged ≥65 years), between the low cut-off and <2.67, 2.67-<3.25, and ≥3.25 in 66.3%, 23.9%, 3.4%, and 6.4% of patients; the corresponding annual HCC incidence was 0.07%, 0.17%, 0.77%, and 1.18%. As a standalone test, the annual HCC incidence exceeded 0.2% for LSM ≥10 kPa and 1% for LSM ≥20 kPa. If LSM was performed as a second step only among patients with FIB-4 above the low cut-off, the annual HCC incidence exceeded 0.2% for LSM ≥10 kPa and 1% for LSM ≥15 kPa.

Conclusion: HCC surveillance should be offered to MASLD patients with FIB-4 ≥3.25 or LSM ≥20 kPa. When two-step approach is adopted, LSM ≥15 kPa in patients with increased FIB-4 predicts a high HCC risk.

What is already known on this topic

Non-invasive tests of fibrosis such as the Fibrosis-4 index (FIB-4) and liver stiffness measurement (LSM) by vibration-controlled transient elastography are associated with the risk of hepatocellular carcinoma (HCC) in patients with metabolic dysfunction-associated steatotic liver disease (MASLD). The test thresholds that should prompt the initiation of HCC surveillance remain unclear.

What this study adds

In this cohort study of 12,950 patients with MASLD, FIB-4 ≥3.25 or LSM ≥20 kPa exceeded the HCC surveillance threshold. Adopting the two-step algorithm to assess LSM in patients classified as increased risk by FIB-4, LSM ≥15 kPa showed a high risk of HCC to justify surveillance.

How this study might affect research, practice or policy

The findings of this study suggest that the two non-invasive tests predict the risk of HCC, and the two-step algorithm can further categorise the risk in patients with MASLD.

INTRODUCTION

Metabolic dysfunction-associated steatotic liver disease (MASLD), previously known as non-alcoholic fatty liver disease, affects over 30% of the general population and has been the most rapidly rising cause of hepatocellular carcinoma (HCC) in the past decade.[1, 2] HCC is often asymptomatic until late stage. Therefore, international guidelines recommend biannual abdominal ultrasonography for HCC surveillance in at-risk populations so that cancer can be diagnosed in an earlier stage when curative-intent treatments can be offered.[3, 4] At-risk population is typically defined based on the incidence of HCC above which HCC surveillance would be cost-effective. For MASLD, current guidelines only recommend surveillance in patients with cirrhosis. However, up to 30-50% of patients with MASLD develop HCC before the onset of cirrhosis.[5, 6] Besides, the absolute incidence of HCC is lower in patients with MASLD than in those with other liver diseases, even among patients with cirrhosis.[7] Furthermore, because MASLD causes hepatomegaly and bright liver echotexture, typical features of cirrhosis such as shrunken liver and nodular surface are less apparent on routine imaging.[8] For these reasons, it is crucial to define the at-risk population in more concrete terms.

A number of non-invasive tests of liver fibrosis have shown good accuracy for the diagnosis of liver fibrosis and prediction of HCC and cirrhotic complications.[9, 10] In particular, international guidelines recommend the use of the Fibrosis-4 index (FIB-4), a simple formula comprising of age, liver enzymes and platelet count, for initial screening of advanced liver fibrosis in patients with MASLD.[11, 12, 13] A second-line specific fibrosis biomarker such as liver stiffness measurement (LSM) by vibration-controlled transient elastography (VCTE) is performed when FIB-4 shows indeterminate results. However, cut-offs for the detection of advanced fibrosis might not be the optimal levels to recommend HCC surveillance.

Using the large multicentre VCTE-Prognosis study cohort, we determined the incidence of HCC at different FIB-4 and LSM levels to facilitate evidence-based recommendations on HCC surveillance. In addition, we evaluated the optimal use of the two-step algorithm for HCC risk prediction.

METHODS

Study design and participants

This cohort study included patients with MASLD who had received VCTE examination at 16 centres across the US, Europe, and Asia, of which data were collected prospectively at 14 centres. Details of the study design have been previously reported.[14] In brief, we included adult patients aged 18 years or older with hepatic steatosis diagnosed by histologic methods (steatosis in ≥5% of hepatocytes) or imaging studies (ultrasonography, computed tomography or magnetic resonance imaging, or controlled attenuation parameter ≥248 dB/m by VCTE) and available FIB-4 and VCTE results. Patients with other liver diseases such as chronic viral hepatitis, HIV infection, excessive alcohol consumption (>30 g/day in men and >20 g/day in women), secondary causes of hepatic steatosis, or history of HCC, hepatic decompensation, liver resection, liver transplant, or other malignancies, were excluded.

The STARD (Standards for Reporting of Diagnostic Accuracy) 2015 guidelines were followed for conduction of the study (Supplementary Table 1).[15] The study was conducted in accordance with the principles of the Declaration of Helsinki with the study protocol approved by the Joint Chinese University of Hong Kong-New Territories East Cluster Clinical Research Ethics Committee (Reference number: 2022.255) and the institutional review boards of the participating sites. The patients provided informed written consent for the prospective programs at the local sites, but consenting for the current secondary analysis was waived. There was no patient or public involvement in the design, conduct, reporting, or dissemination plans of our research.

Study assessments

The investigators recorded the medical history at each clinic visit. Body mass index (BMI) was calculated as weight (kg) divided by height (m) squared. A venous blood sample was taken after at least 8 hours of fasting for complete blood count, liver biochemistry, kidney function, glucose, and lipids. FIB-4 was calculated as age (years) × aspartate aminotransferase (AST, U/L)/ [Platelets (10^9/L) × alanine aminotransferase (ALT)$^{1/2}$ (U/L)].[16] Liver stiffness was measured using the VCTE machine (FibroScan, Echosens, Paris, France) by trained operators, with at least 10 valid acquisitions.[17]

Definition of non-invasive approaches

In this study, we utilised FIB-4 and LSM to categorise patients into distinct risk groups and examine the annual incidence of HCC across these classifications. For FIB-4, the cut-offs applied were 1.3 for individuals younger than 65 years, 2.0 for those aged 65 and older, followed by 2.67 and 3.25.[12] The LSM cut-offs (in kPa) were set at 8, 10, 12, 15, 20, and 25 in line with those used in various guidelines to detect advanced liver fibrosis, cirrhosis and clinically significant portal hypertension.[11, 18] We employed the FIB-4 and the LSM cut-offs separately for HCC risk stratification. Additionally, we investigated the proposed two-step algorithm: first, patients were divided into two groups using the FIB-4 threshold of 1.3 (for age <65 years) or 2.0 (for age ≥65 years). Those with FIB-4 exceeding this threshold were further classified using the LSM cut-offs. Besides, we also use a threshold of 2.67 for FIB-4 to implement the two-step algorithm.

Outcomes

The primary outcome was incident HCC. The diagnosis of HCC was based on prospective follow-up, medical record review, or validated registries with positive predictive values (PPVs) of at least 90% and in accordance with international guidelines.[3]

Statistical analysis

The baseline date was defined as the date of first VCTE and blood tests, whichever later, to avoid immortal time bias, as previously described.[14] Patients were followed until the development of HCC, death, or date of the last follow-up, whichever came first; non-HCC-related death was considered a competing risk. Data were analysed using R (4.4.0, R Core Team 2024). R packages "survival", "cmprsk", "timeROC", "rms", and "dcurves" were used in the analysis. Continuous variables were expressed in mean (standard deviation) or median (25^{th} to 75^{th} percentile [P25-P75]), as appropriate, while categorical variables were presented as number (percentage). The cumulative incidence function of HCC was estimated and compared by Gray's test; non-HCC-related death was treated as a competing event for HCC.[14] The discriminatory performance of non-invasive approaches was assessed by time-dependent area under the receiver-operating characteristic curves (AUROCs) accounting for competing events.[19] Integrated time-dependent AUROC summarised the time-dependent AUROCs over 5 years of follow-up, calculated as an average of time-dependent AUROCs weighted by the estimated probability density of HCC during

follow-up; 95% confidence interval (CI) was estimated using nonparametric bootstrapping with 1,000 bootstrap samples. Calibration plots were used to assess the calibration of non-invasive approaches. The predicted risk of HCC in the calibration plots referred to the estimated probability of developing HCC by the non-invasive approaches based on the Fine and Gray subdistribution hazard model. Time-dependent sensitivity, specificity, PPV, and negative predictive value (NPV) at 3 and 5 years were evaluated, accounting for competing risks based on Fine and Gray's method. Decision curve analysis was used to assess the clinical benefit of using non-invasive approaches to inform the decision of HCC surveillance. It was performed based on the estimated incidence of HCC at 5 years by the non-invasive approaches using Fine and Gray subdistribution hazard model. Subgroup analyses were performed among patient subgroups stratified by age, sex, presence of diabetes and BMI. All statistical tests were two-sided. Statistical significance was taken as $p<0.05$.

Role of the funding source

The funder of the study did not have a role in study design, data collection, data analysis, data interpretation, or manuscript preparation. Echosens provided logistic support in contacting investigators and organising investigator meetings but did not provide funding for this study.

RESULTS

Participants

From February 2004 to January 2023, 17,949 patients who underwent one or more VCTE examinations were identified. After excluding 4,999 patients (679 patients younger than 18 years old or with unknown age, 598 patients with HCC or hepatic decompensation before VCTE or lacking follow-up data, 69 with HCC or hepatic decompensation within 3 months after VCTE, and 3,653 without available FIB-4), a total of 12,950 patients with MASLD and available LSM and FIB-4 were included in the final analysis (**Figure 1**). The mean (SD) age of these patients was 51.7 (13.9) years with 5,316 females (41.1%) (**Table 1**). 4,429 (34.2%) patients had diabetes.

Among the 12,950 patients, 66.3% had a FIB-4 below the cut-off of 1.3 (for age <65 years) or 2.0 (for age ≥65 years). 9.8% of patients had FIB-4 ≥2.67 and 6.4% had FIB-4 ≥3.25 (Supplementary Figure 1A). 72.7% of patients had LSM below 8 kPa, while 8.1% of patients had LSM ≥15 kPa and 3.0% had LSM ≥25 kPa (Supplementary Figure 1B).

Overall incidence of HCC

At a median (P25-P75) follow-up of 47.4 (23.3 to 72.3) months, 109 (0.8%) patients developed HCC. The overall cumulative incidence of HCC for 12,950 patients was 0.5% (95% confidence interval [CI] 0.4%-0.6%) at 3 years and 1.0% (95% CI 0.8%-1.2%) at 5 years (Supplementary Figure 2).

Incidence of HCC among different groups classified by FIB-4

Based on the three FIB-4 cut-offs of 1.3 (for age <65 years) or 2.0 (for age ≥65 years), 2.67, and 3.25, patients were divided into four categories, with the corresponding annual incidence of HCC being 0.07%, 0.17%, 0.77%, and 1.18%, respectively (**Figure 2A**). For patients with FIB-4 below 1.3 (for age <65 years) or 2.0 (for age ≥65 years), the 5-year cumulative incidence of HCC was 0.3% (95% CI 0.2%-0.5%). For those with FIB-4 between 1.3 (for age <65 years) or 2.0 (for age ≥65 years) and 2.67, the 5-year cumulative incidence was 0.9% (95% CI 0.5%-1.4%). Patients with FIB-4 between 2.67 and 3.25 had a 5-year cumulative incidence of 3.9% (95% CI 2.0%-6.7%), while those with FIB-4 above 3.25 had a 5-year cumulative incidence of 5.9% (95% CI 4.0%-8.2%) (p<0.001) (**Figure 3A**). Similar patterns were observed in subgroup analyses based on age, BMI and the presence of diabetes, except for females who had an annual incidence of HCC <1% in all four FIB-4 categories (Supplementary Figure 3A).

Incidence of HCC among different groups classified by LSM

Patients were divided into seven groups according to the six LSM cut-offs of 8, 10, 12, 15, 20, and 25 kPa, with the annual incidence of HCC being 0.06%, 0.07%, 0.27%, 0.53%, 0.86%, 1.11% and 1.55% respectively (**Figure 2B**). For patients with LSM below 10 kPa, the 5-year cumulative incidence of HCC was 0.3% (95% CI 0.2%-0.5%). The 5-year cumulative incidence of patients with LSM exceeding 10 kPa ranged from 1.3% to 7.8% (**Figure 3B**). Similar patterns were observed in subgroup analyses based on age and the presence of diabetes, except for females which had an annual incidence of HCC <1% in all seven LSM categories. Patients with BMI <30 kg/m^2 had <1% annual incidence of HCC while LSM was ≥20-<25 kPa, and 2.2% while LSM was ≥25 kPa. For patients with BMI ≥30 kg/m^2, the annual incidences of HCC were 1.5% and 1.0% for LSM ≥20-<25 kPa and ≥25 kPa, respectively (Supplementary Figure 3B).

Incidence of HCC among different groups classified by two-step algorithm

Following the two-step algorithm, we further classify the patients with FIB-4 over 1.3 (for age <65 years) or 2.0 (for age ≥65 years) into seven groups using the cut-offs of LSM. The annual incidence of HCC ranged from 0.09% to 0.42% for those with LSM <12 kPa and from 0.65% to 1.89% for those with LSM ≥12 kPa (**Figures 2C, 3C**). Similar patterns were observed in subgroup analyses based on age and the presence of diabetes except for females which had an annual incidence of HCC <1%. The annual incidence of HCC was <1% for patients with BMI <30 kg/m^2 when LSM was ≥20-<25 kPa while that exceeded 1% when LSM was ≥15-<20 kPa or ≥25 kPa. When BMI was ≥30 kg/m^2, the annual incidences of HCC were 1.3%, 1.7% and 1.2% when LSM were ≥15-<20 kPa, ≥20-<25 kPa and ≥25 kPa, respectively (Supplementary Figure 3C). In addition, if a FIB-4 cut-off of 2.67 was used instead as the first step, the annual incidence of HCC ranged from 0.22% to 0.94% for those with LSM <12 kPa, and 1.16% to 2.42% for those with LSM ≥12 kPa (Supplementary Figures 3D and 4-5). **Figure 4** and Supplementary Figure 6 illustrate the two-step algorithm using FIB-4 of 1.3 (for age <65 years) or 2.0 (for age ≥65 years) and 2.67, respectively as the first assessment.

Performance of two-step algorithm in prediction of HCC

The two-step algorithm using FIB-4 over 1.3 (for age <65 years) or 2.0 (for age ≥65 years) and LSM ≥15 kPa had an overall specificity of 93.8% (95% CI 93.3%-94.3%) and PPV of 3.7% (95% CI 2.4%-5.1%) to predict HCC at 3 years. The specificity increased to 94.4% (95% CI 93.9%-94.9%) with a PPV of 7.9% (95% CI 5.7%-10.5%) for the prediction of HCC at 5 years. Whereas, for LSM <15 kPa despite FIB-4 >1.3/2.0, the sensitivity was 48.4% (95% CI 34.6%-63,2%) with a NPV of 99.7% (95% CI 99.6%-99.8%) for HCC at 3 years, with similar findings at 5 years. The AUROC was 0.733 (95% CI 0.666-0.795) with an overall accuracy above 93% (Supplementary Table 2 and Supplementary Figures 7-9).

DISCUSSION

In this large multicentre cohort study, we found that high FIB-4 and LSM were associated with an increased risk of HCC in patients with MASLD. Adopting the two-step algorithm allowed to screen out patients at low risk of HCC while at the same time further stratifying the risk among

those with increased risk by FIB-4 (i.e., ≥1.3 [for age <65 years] or 2.0 [for age ≥65 years]), and those with LSM ≥15 kPa had a substantial risk of HCC in which regular surveillance of HCC should be considered.

With the emerging population of MASLD around the world, the number of patients with MASLD-related HCC will rise.[20, 21] Guidelines have suggested surveillance for HCC if the annual incidence of HCC is above 1%, implying that patients with MASLD-related cirrhosis should be regularly monitored for HCC.[3, 22] Cirrhosis is, strictly speaking, a histological diagnosis but liver biopsy has been obsolete for pure diagnostic purpose outside research and clinical trial settings.[23] Non-invasive tests have been employed to assess the degree of liver fibrosis but there is a lack of concrete recommendations on the thresholds of non-invasive test(s) that suggest the need for HCC surveillance. A recent study by Gu *et al.* proposed the PLEASE algorithm, considering age, sex, platelet count, LSM by two-dimensional shear wave elastography and aetiology of liver disease (steatotic liver disease and viral hepatitis) as risk factors, to stratify the risk of HCC in patients with advanced chronic liver disease.[24] However, this was not intended to inform thresholds of common non-invasive tests that would warrant HCC surveillance in patients with MASLD. Nonetheless, the common constituents of this algorithm and FIB-4 echoed the role of this simple first-line non-invasive test in determining the risk of HCC and hence the surveillance threshold.[12, 13, 25] With the low FIB-4 cut-off of 1.3 (for age <65 years) or 2.0 (for age ≥65 years) showing high sensitivity and negative predictive value for advanced liver fibrosis, and the high cut-offs of 2.67 and 3.25 suggesting a high specificity and positive predictive value for advanced liver fibrosis and/or cirrhosis, respectively,[26, 27] our findings confirmed the minimal risk of HCC among those in the low-risk group, whereas the annual incidence was 1.18% among those with FIB-4 ≥3.25, reinforcing current guideline recommendations to provide surveillance of HCC.

VCTE has been widely utilized to assess LSM which correlates well with the degree of liver fibrosis and portal hypertension. LSM ≥15 kPa indicates the presence of compensated advanced chronic liver disease (cACLD) which consists of the spectrum from advanced liver fibrosis to compensated cirrhosis.[18, 28] As a standalone test, we showed that LSM ≥15-<20 kPa conferred a 0.86% annual incidence of HCC and that increased to at least 1.11% for those with LSM ≥20

kPa. However, if one preselects patients with increased FIB-4 in accordance with current guideline recommendations,[12, 13, 25] patients with LSM ≥15 kPa had an annual incidence of HCC exceeding 1.0%, thus indicated for HCC surveillance. Notably, our subgroup analyses based on age and the presence of diabetes showed similar trends in the risk of HCC with regard to the cut-off values of FIB-4 and LSM. This adds value to the universal adoption of VCTE assessment as a two-step algorithm for further risk stratification when FIB-4 is above the low cut-off. Otherwise, our subgroup analyses suggested that female patients had a lower incidence of HCC and thus the two-step algorithm to show both high-risk FIB-4 and LSM before initiating HCC surveillance in this subgroup should be warranted. The performance of LSM was less consistent in the obese population. This could be related to the confounding effect of BMI on LSM and more parameter(s) (e.g., platelet count) may be required to demonstrate a more accurate HCC risk pattern across different LSM thresholds.[18, 29] Despite that, the LSM cut-offs as standalone test or in two-step approach to initiate HCC surveillance in different BMI generally followed the main analysis and thus the same LSM cut-offs were applicable.

Furthermore, a proportion of patients with MASLD do not have cirrhosis when HCC is diagnosed. In prior studies, non-cirrhotic MASLD translated to a negligible risk of HCC development.[30, 31] However, within the wide spectrum of non-cirrhotic patients, it is important to identify the subgroup of patients at risk of developing HCC. This was echoed by previous observational studies showing that up to 30%-50% of patients with MASLD-related HCC were non-cirrhotic.[5, 32, 33] This notion proposed the potential need to expand the population among patients with MASLD to receive HCC surveillance and a clear risk stratification on the subgroup of non-cirrhotic patients to be screened for HCC. Our current study answered this by showing that non-cirrhotic patients with cACLD based on LSM criteria should be put on regular HCC surveillance. Consequently, adopting the two-step algorithm will identify patients at risk of HCC development for further care by hepatologists with regular surveillance for HCC while the low-risk group will receive interval assessments with non-invasive tests to detect for any interval progression. This is particularly reinforced by the high accuracy of the two-step algorithm in predicting HCC given a lower incidence rate of HCC overall. This allows better resource allocation and enhances the clinical care of patients with MASLD.[34]

HCC surveillance is suggested for non-cirrhotic chronic hepatitis B patients when the annual incidence of HCC reaches ≥0.2%.[35] Lowering the threshold for HCC surveillance in patients with MASLD in line with that of chronic hepatitis B will lead to an increase in the absolute number of HCC detected but at the same time lead to a significant surge in healthcare burden due to many patients needed for HCC surveillance. Our study reinforces the importance of two-step algorithm in identifying patients at risk of HCC which lays a foundation for future dedicated cost-effectiveness analysis, especially with the changing epidemiology and prevalence of patients with MASLD globally. The cost-effectiveness of HCC surveillance depends on the local healthcare system and the costs of various tests and treatments, and our study provides granular data on non-invasive test cut-offs should a country prefer starting surveillance at a different HCC incidence threshold.

Our study has the strength of having a multicentre cohort with different ethnicities to enhance the generalizability of the study results. This study design with a large sample size allows an adequate number of incident HCC cases to carry out a comprehensive analysis. With that, the study findings acknowledge the current clinical guidelines on the two-step algorithm in managing patients with MASLD. On the other hand, our study carries a few limitations. First, the 47-month median follow-up is relatively short given the long duration required in MASLD to have disease progression and HCC.[36] Nonetheless, the large sample size has partly offset this constraint with an adequate event rate. Besides, current guidelines recommend fibrosis assessment at intervals of 1 to 3 years in patients with MASLD, and risk prediction should be updated accordingly. Secondly, our analyses were based on the FIB-4 and LSM measured at baseline. Whether serial changes of these parameters can suggest any additional risk of HCC or termination of HCC surveillance is uncertain and should be addressed in future studies. By the same token, whether treatment for metabolic dysfunction-associated steatohepatitis would alter the natural history and HCC incidence remains to be ascertained.

CONCLUSIONS

In this cohort study, the two non-invasive tests, FIB-4 and LSM from VCTE, were found to be predictive of the risk of HCC development among patients with MASLD. HCC surveillance should be offered to MASLD patients with FIB-4 ≥3.25 or LSM ≥20 kPa. When a two-step approach is

adopted, LSM ≥15 kPa in patients with increased FIB-4 predicts a high risk of HCC. The findings suggest that the currently suggested non-invasive tests can stratify the risk and thus guide surveillance for HCC, further streamlining the management of patients with MASLD.

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Table 1. Clinical characteristics for all 12,950 patients with metabolic dysfunction-associated steatotic liver disease (MASLD).

Clinical characteristics	**All patients N = 12,950**
Age (years)	51.7 (13.9)
Male sex, n (%)	7,634 (58.9)
BMI (kg/m^2)	27.2 (24.7 – 30.4)
Comorbidities, n (%)	
Diabetes	4,429 (34.2)
Hypertension	4,835 (37.3)
Laboratory parameters	
ALT (IU/L)	38 (24 – 64)
AST (IU/L)	31 (23 – 47)
GGT (IU/L)	44 (27 – 78)
Albumin (g/L)	44.8 (3.8)
Total bilirubin (mg/dL)	0.70 (0.50 – 0.94)
Platelet (×10^9/L)	239 (200 – 282)
Creatinine (mg/dL)	0.81 (0.68 – 0.94)
Non-invasive tests results	
Liver stiffness measurement by VCTE (kPa)	5.9 (4.6 – 8.3)
LSM <8 kPa, n (%)	9,420 (72.7)
LSM ≥8 kPa, n (%)	3,530 (27.3)
LSM ≥10 kPa, n (%)	2,329 (18.0)
LSM ≥12 kPa, n (%)	1,629 (12.6)
LSM ≥15 kPa, n (%)	1,044 (8.1)
LSM ≥20 kPa, n (%)	620 (4.8)
LSM ≥25 kPa, n (%)	390 (3.0)
Controlled attenuation parameter (dB/m)	303 (274 – 335)
Fibrosis-4 index (FIB-4)	1.11 (0.74 – 1.71)
FIB-4 <1.3 for age <65 years or <2.0 for age ≥65 years, n (%)	8,582 (66.3)
FIB-4 ≥1.3 for age <65 years or ≥2.0 for age ≥65 years, n (%)	4,368 (33.7)
FIB-4 ≥2.67, n (%)	1,272 (9.8)
FIB-4 ≥3.25, n (%)	832 (6.4)
Follow-up duration (months)	47.4 (23.3 – 72.3)

Data are presented as n (%), mean (standard deviation), or median (25th – 75th percentile), as appropriate.

Liver stiffness measurement is a non-invasive method to evaluate liver fibrosis, using transient elastography to measure liver stiffness, which helps in assessing the extent of fibrosis; Controlled attenuation parameter quantifies liver steatosis non-invasively, by measuring the attenuation of ultrasound waves through the liver, providing an indicator of fat levels.

Abbreviations: AST, aspartate aminotransferase. ALT, alanine aminotransferase. BMI, body mass index. FIB-4, Fibrosis-4 index. GGT, gamma-glutamyl transpeptidase. VCTE, vibration-controlled transient elastography.

FIGURE LEGENDS

Figure 1. Study participant flowchart.

Figure 2. The annual incidence of hepatocellular carcinoma (HCC) in patients under different risk categories as defined by A. fibrosis-4 index (FIB-4), B. liver stiffness measurement (LSM), and C. LSM among patients with FIB-4 ≥1.3 (age <65 years) or FIB-4 ≥2.0 (age ≥65 years).

Figure 3. Cumulative incidence of hepatocellular carcinoma (HCC) in patients under different risk categories as defined by A. fibrosis-4 index (FIB-4), B. liver stiffness measurement (LSM), and C. LSM among patients with FIB-4 ≥1.3 (age <65 years) or FIB-4 ≥2.0 (age ≥65 years).

Figure 4. Two-step algorithm flowchart using fibrosis-4 index (FIB-4) cutoff of 1.3 (age <65 years) or 2.0 (age ≥65 years) followed by liver stiffness measurement by vibration-controlled transient elastography in patients with increased FIB-4.