The occurrence of hepatocellular carcinoma in different risk stratifications of clinically noncirrhotic nonalcoholic fatty liver disease

Teng-Yu Lee[1,2], Jaw-Ching Wu[3,4], Shi-Hang Yu[1], Jaw-Town Lin[5,6,7], Ming-Shiang Wu[8] and Chun-Ying Wu [1,9,10,11]

[1] Division of Gastroenterology & Hepatology, Taichung Veterans General Hospital, Taichung, Taiwan

[2] Department of Medicine, Chung Shan Medical University, Taichung, Taiwan

[3] Institute of Clinical Medicine and Genomic Research Center, National Yang-Ming University, Taipei, Taiwan

[4] Translational Research Division, Medical Research Department, Taipei Veterans General Hospital, Taipei, Taiwan

[5] School of Medicine, Fu Jen Catholic University, New Taipei City, Taiwan

[6] Department of Internal Medicine, E-Da Hospital/I-Shou University, Kaohsiung, Taiwan

[7] Center for Health Policy Research and Development, National Health Research Institutes, Miaoli, Taiwan

[8] Department of Internal Medicine, National Taiwan University Hospital, Taipei, Taiwan

[9] Faculty of Medicine, School of Medicine, National Yang-Ming University, Taipei, Taiwan

[10] College of Public Health and Graduate Institute of Clinical Medical Science, China Medical University, Taichung, Taiwan

[11] Department of Life Sciences, National Chung Hsing University, Taichung, Taiwan

Nonalcoholic fatty liver disease (NAFLD) may be a cause of hepatocellular carcinoma (HCC), but its high prevalence challenges current surveillance strategies. We aimed to evaluate HCC incidences in different risk stratifications for noncirrhotic NAFLD. Using Taiwan's National Health Insurance Research Database, we located 31,571 patients with NAFLD between the years 1998 and 2012. After excluding other causes of hepatitis, underlying cirrhosis or malignancy, 18,080 patients were recruited for final analysis. Cumulative incidences of HCC were analyzed after adjusting for competing mortality. With a median follow-up duration of 6.32 years in the study cohort, the 10-year cumulative incidence of HCC was 2.73% [95% confidence interval (CI): 1.69–3.76%]. Hepatoprotectant was used as a surrogate marker for elevated serum alanine transaminase (ALT). After adjusting for age, gender, hypertension, hypercholesterolemia, diabetes mellitus, gout, statin use, metformin use and aspirin use, elevated ALT was independently associated with an increased HCC risk [hazard ratio (HR) 6.80, 95% CI: 3.00–15.42; $p < 0.001$]. Multivariate stratified analysis verified this association in all subgroups (HR> 1.0). Moreover, increased age (HR 1.08 per year, 95% CI: 1.05–1.11) and statin use (HR 0.29, 95% CI: 0.12–0.68) were also identified as independent risk factors. The 10-year cumulative HCC incidence was highest in older (age >55 years) patients with ALT elevation (12.41%, 95% CI: 5.99–18.83%), but lowest in younger patients without ALT elevation (0.36%, 95% CI: 0–1.08%). The incidence of HCC was relatively low in patients with clinically non-cirrhotic NAFLD, however, HCC risk was significantly increased in older patients experiencing an elevated serum ALT.

The prevalence of nonalcoholic fatty liver disease (NAFLD) is rising worldwide, at an estimated rate of 24%, according to surveys performed between 2005 and 2014.[1] NAFLD may possibly evolve into liver necroinflammation, fibrosis, cirrhosis and/or hepatocellular carcinoma (HCC).[2] The number of

NAFLD-related HCC incidents has been reported to be increasing annually by 9% in the United States, and NAFLD may become a major cause of HCC upon the introduction of direct acting antiviral drugs used to fight viral hepatitis.[3,4] Among patients with NAFLD-related liver cirrhosis, the

What's new?

The prevalence of nonalcoholic fatty liver disease (NAFLD) is rising worldwide; however, risk-oriented tumor surveillance guidelines for noncirrhotic NAFLD patients is lacking. In this population-based cohort study, the 10-year cumulative incidence of liver cancer among noncirrhotic NAFLD patients was low overall (2.7%). However, marked differences were found among older patients with elevated serum alanine transaminase levels (highest incidence) and younger patients without liver enzyme elevation (lowest incidence). This points to the usefulness of tumor surveillance in older NAFLD patients with or without liver enzyme abnormalities.

yearly cumulative incidence of HCC is quite high,[5] therefore, regular HCC surveillance of cirrhotic patients has been recommended under the current guidelines.[6–8] However, among NAFLD patients who are not experiencing underlying cirrhosis, the incidence of HCC has been estimated to be only 1–3% over the course of 10 years,[9,10] yet a clear follow-up strategy for NAFLD patients without cirrhosis is lacking. The high prevalence of NAFLD may contribute to an increased numbers of HCC cases in developed countries,[11] however, extensive surveillance of all NAFLD patients may not be either practical or cost-effective.[8] Identifying a high-risk subpopulation in HCC development among noncirrhotic NAFLD patients is imperative.

Although a liver biopsy remains the gold standard when evaluating the severity of NAFLD, this invasive procedure is not a suitable option as a routine screening tool for this common disease.[8,12] For easy identification of noncirrhotic NAFLD patients who are at an especially high risk of HCC development, an easy and inexpensive noninvasive test to predict prognosis needs to be discovered. Serum alanine aminotransferase (ALT) has been widely used as a surrogate marker for liver damage, however, several cross-sectional studies have found that a normal ALT value does not guarantee a patient is free from either steatohepatitis or advanced fibrosis.[13,14] ALT levels can fluctuate during the follow-up period, so their accuracy may be affected by the timing of when a test was performed.[15,16] However, a higher ALT level is associated with a higher activity of steatohepatitis in NAFLD,[17,18] while the long-term outcome in NAFLD patients with an elevated ALT may be different from those without an elevated ALT. Therefore, a longitudinal study evaluating the risk of ALT elevation on HCC development is mandatory.

Several potential risk factors related to HCC development in NAFLD, such as advanced fibrosis, older age and male gender, have been reported in previous investigations[8,11]; however, longitudinal studies used to evaluate HCC incidences within different risk levels of noncirrhotic NAFLD patients remain sparse. In this study, we aimed to conduct a large-scale, long-term cohort investigation to evaluate the cumulative incidence of HCC in different risk stratifications of NAFLD patients who have not been clinically diagnosed with cirrhosis.

Materials and Methods
Study design
In this population-based retrospective cohort study, we retrieved medical records from the Longitudinal Health Insurance Databases 2000 and 2005 (LHID2000 and LHID2005). These contained 2,000,000 randomly sampled subjects from Taiwan's National Health Insurance Research Database (NHIRD), between January 1, 1998 and December 31, 2012. Any subjects who had been repeatedly sampled in LHID2000 and LHID2005 were excluded. The NHIRD contains healthcare data from >99% of Taiwan's entire population of 23.38 million.[19] The database is comprised of comprehensive information, including demographic data, diagnostic codes, dates of clinic visits and hospitalizations, along with details of prescriptions among other data, as outlined in our previous studies.[20–23] The NHIRD uses the International Classification of Diseases, 9th Revision (ICD-9) codes to define diseases. The Research Ethics Committee of the National Health Research Institutes in Taiwan approved our study.

Study population
The process of patient selection is shown in Figure 1. We included all 31,571 patients with NAFLD (ICD-9 code: 571.8) who had been diagnosed at least three times in outpatient clinics, or one time during hospitalization between January 1, 1998 and December 31, 2012. Patients with an age <18 years at the index diagnosis of NAFLD were excluded. After we excluded patients who were diagnosed with other causes of their hepatitis, including alcoholic liver disease (ICD-9 codes 571.0–3, 303.9, V11.3 and V79.1), any viral hepatitis (ICD-9 codes 070, 573.1, V02.6 and V05.3), human immunodeficiency virus (HIV) infection (ICD-9 codes 042), other infectious hepatitis (ICD-9 codes 573.2), toxic hepatitis (ICD-9 codes 573.3), biliary cirrhosis (ICD-9 codes 571.6) and autoimmune hepatitis (ICD-9 codes 571.42) throughout the whole database (from March 1, 1995 to December 31, 2012), a total of 20,885 patients with NAFLD were then selected. In order to avoid any observational bias, we further excluded patients who did not call for a medical visit at least one time in a year during the study period. In addition, patients experiencing underlying cirrhosis (ICD-9 codes 571.5, 572.2, 572.4 and 789.5) or any malignancy (ICD-9 codes 140–208) before or within 180 days after the index diagnosis of NAFLD, were also excluded. A total of 18,080 NAFLD patients were eventually recruited for final analysis.

Main outcome measurements
Patients in the study cohort were followed up after the index dates of their NAFLD diagnosis, until their dates of mortality during the study period or the end of the study period
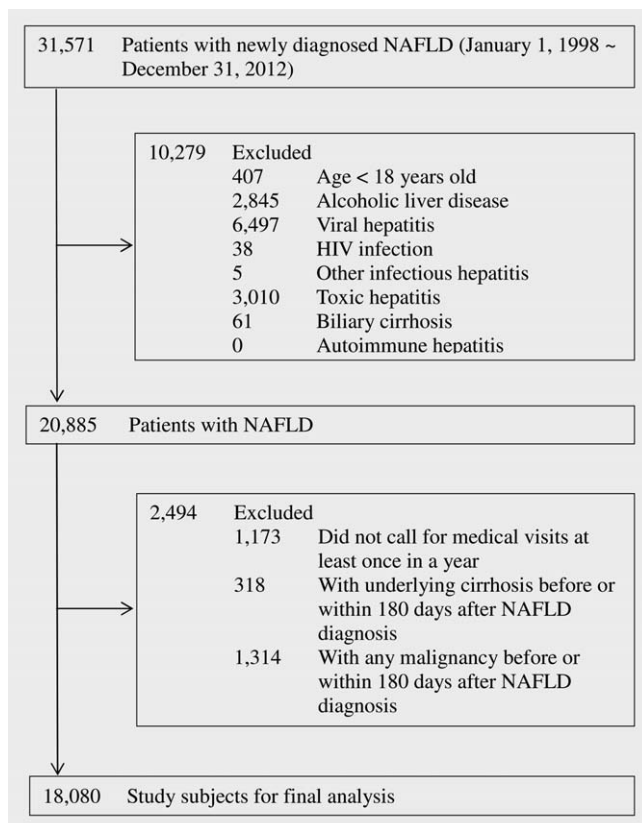
Figure 1. Selection of study subjects.

(December 31, 2012). HCC development was the main outcome measurement of our study, and all patients who were admitted with a primary diagnosis of HCC (ICD-9 code 155.0) were identified. We further confirmed the validity of HCC diagnosis through the inclusion of patients from the Registry for Catastrophic Illness Patient Database (RCIPD). The RCIPD is an official sub-system within the NHIRD, where histopathological confirmation or typical dynamic image characteristics of HCC are required for the diagnosis of HCC.[20–22] Based on the practice guidelines, only patients without typical dynamic image presentation are recommended for histopathological confirmation.[6–8]

Risk factor assessment

In addition to logging each patient's age and gender, major coexisting diseases which may increase the risk of HCC development were also evaluated at the index diagnosis of NAFLD, including hypertension (ICD-9 code 401–405), diabetes mellitus (ICD-9 code 250), hypercholesterolemia (ICD-9 code 272.0, 272.2) and gout (ICD-9 code 274). Furthermore, information pertaining to drug use was retrieved from the database of pharmaceutical prescriptions. In order to evaluate the effects of ALT elevation on HCC development, we used a prescription of hepatoprotectants (*e.g.*, silymarin, liver hydrolysate and choline bitartrate) to be used as a surrogate marker of ALT elevation, as hepatoprotectant

reimbursement requires patients to fulfill the criteria of an ALT elevation $\geq 2\times$ the upper limits of normal (ULN). In addition, some chemopreventive medications, including statins, metformin and aspirin, were also analyzed as potential risk factors. The users of these drugs were defined as the patients who took them >1 day per month during the outcome follow-up period.

Statistical analysis

Continuous variables were presented as median values (25–75% interquartile ranges). Accordingly, case numbers along with their percentages (%) were used to describe study subjects in the study cohort. Multivariate regression analyses for age, gender, ALT elevation, hypertension, hypercholesterolemia, diabetes mellitus, gout, statin use, metformin use and aspirin use were conducted to determine any independent risk factors for HCC development; while hazard ratios (HRs) were determined by Cox proportional hazard models. To avoid immortal time bias, we treated the use of medications during the study period as the time-dependent variable in Cox proportional hazards models.[24] Multivariate stratified analysis of the effects of the risk factor was also performed in patient subgroups. Cumulative incidences of HCC during the study period were calculated by use of the modified Kaplan–Meier method after adjusting for competing mortality risks, where the calculated rates were expressed as the estimated number along with the 95% confidence interval (CI). According to the findings of the multivariate regression analyses, the cumulative incidences of HCC among patients with different risk factors were, respectively, estimated and compared to each other. All data were analyzed through the use of SAS 9.3 software (SAS Institute, Inc., Cary, NC, USA).

Results

Study subjects

The baseline demographic characteristics of the study subjects are shown in Table 1. The median age was 52.69 years, and >75% of the patients were 65 years of age or younger. Males numbered slightly more than females. The median duration of follow-up was 6.32 (25–75% interquartile range: 3.04–10.10) years. Approximately 22% of the patients received hepatoprotectant due to their ALT elevation. >55%, 37%, 23% and 18% of the patients had underlying hypertension, diabetes mellitus, hypercholesterolemia and gout, respectively. In addition, >28%, 35% and 30% of patients used metformin, statins and aspirin, respectively, during the study period.

Cumulative incidences of HCC in the whole cohort

Cumulative incidences of HCC within the whole cohort are presented in Figure 2a. The 1-year, 3-year, 5-year and 10-year cumulative incidences were found to be only 0.18% (95% CI: 0–0.38), 0.80% (95% CI: 0.37–1.24), 1.03% (95% CI: 0.53–1.54) and 2.73% (95% CI: 1.69–3.76), respectively.

Table 1. Baseline characteristics of the study subjects

Characteristics	$n = 18,080$
Age, years	52.7 (41.6–64.4)[1]
Age, n (%)	
18–45	5,751 (31.8)
46–55	4,335 (24.0)
56–65	3,673 (20.3)
>65	4,321 (23.9)
Sex, n (%)	
Male	9,502 (52.6)
Female	8,578 (47.4)
Follow-up duration (years)	6.32 (3.0–10.1)[1]
ALT elevation during the study period,[2] n (%)	3,956 (21.9)
Major coexisting diseases at baseline, n (%)	
Hypertension	10,107 (55.9)
Diabetes mellitus	6,694 (37.0)
Hypercholesterolemia	4,215 (23.3)
Gout	3,265 (18.1)
Drug users during the study period, n (%)	
Metformin	5,155 (28.5)
Statins	6,382 (35.3)
Aspirin	5,602 (31.0)

[1]Value is presented as a median (25–75% interquartile range). [2]The prescription of hepatoprotectants was used as a surrogate marker of ALT elevation.

Cancer Epidemiology

Multivariate analysis of risk factors

The multivariate regression analysis for determining any independent risk factors for HCC development is presented in Table 2. Compared to patients in the youngest (18–45) year range, the risk of HCC development was shown to be increasing in older aged subjects: 46–55 years (HR 7.25; 95% CI, 0.84–62.87), 56–65 years (HR 21.84; 95% CI, 2.76–172.94) and >65 years (HR 37.07; 95% CI, 4.76–288.69). The HCC risk significantly increased after 55 years of age. When we stratified our study subjects by an age >55 years, those aged >55 years were significantly associated with an increased risk of HCC development (HR 7.78; 95% CI, 3.121–19.44; $p < 0.001$), compared to those who were <55 years of age. When age was evaluated as a continuous variable, an increased age (HR 1.05 per year, 95% CI: 1.04–1.11) was found to be independently related to an increased risk of HCC development. In addition, ALT elevation was another independent factor associated with an increased risk of HCC development (HR 6.80; 95% CI, 3.00–15.42; $p < 0.001$). In contrast, the use of statins (HR 0.29; 95% CI, 0.12–0.68) was an independent factor associated with a decreased risk of HCC development.

Multivariate stratified analysis for ALT elevation

Multivariate stratified analysis for the effect of ALT elevation was performed within subgroups of the patients. These
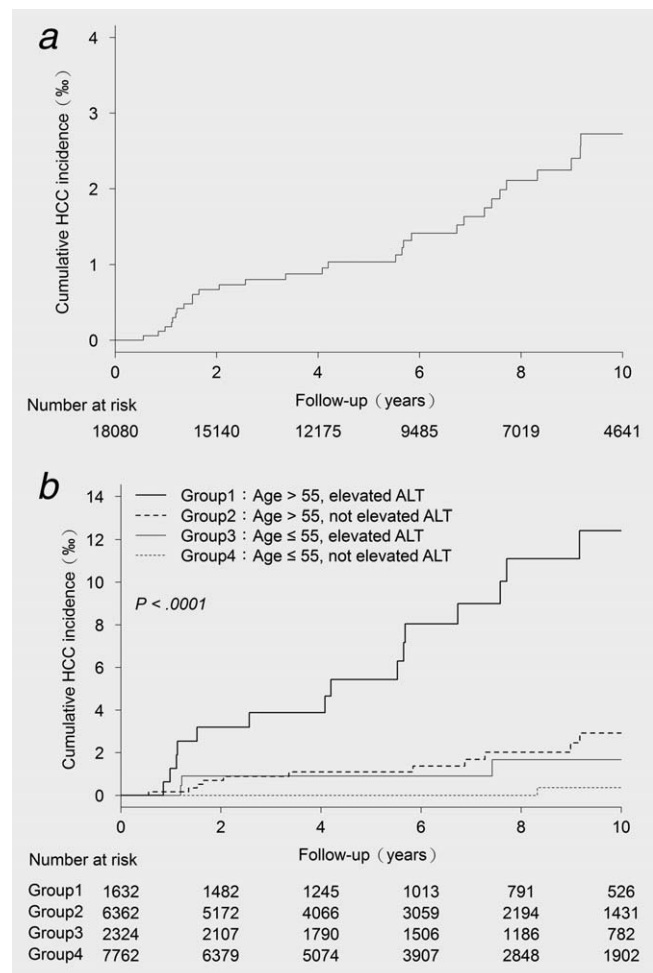

Figure 2. Cumulative incidences of HCC (*a*) in the whole cohort and (*b*) in different risk stratifications of clinically noncirrhotic NAFLD.

included those aged ≤55 or >55 years, both genders, those with underlying hypertension, hypercholesterolemia, diabetes mellitus or gout and finally those who used statins, metformin or aspirin. As shown in Figure 3, multivariate stratified analyses verified the association of ALT elevation with an increased HCC risk within each patient subgroup (HR all >1.0). These findings further confirmed the association between ALT elevation and an increased HCC risk. Furthermore, in regards to the association between ALT elevation and increased HCC risk, statistical significance was reached in nearly all the subgroups. These included patients at any gender, those with or without hypertension, those without hypercholesterolemia, those with or without diabetes mellitus, those without gout, those who did or did not use statins, those who did not use metformin and finally those who did not use aspirin.

Cumulative incidences of HCC in different risk stratifications

We stratified our study subjects according to two key risk factors related to HCC development, *e.g.*, age and ALT

Table 2. Multivariate Cox proportional hazards model analysis for risk of HCC development

Variables	Case no.	HCC no.	HR (95% CI)	p value[1]
Age (years)				
18–45	5,751	1	1	–
46–55	4,335	5	7.25 (0.84–62.87)	0.072
56–65	3,673	12	21.84 (2.76–172.94)	0.003
>65	4,321	23	37.07 (4.76–288.69)	<0.001
Age >55 years	7,994	35	7.78 (3.12–19.44)	<0.001
Age (year)	18,080	41	1.08 (1.05–1.11)	<0.001
Male gender	9,502	25	1.87 (0.99–3.56)	0.055
ALT elevation[2]	3,956	24	6.80 (3.00–15.42)	<0.001
Hypertension[3]	10,107	34	1.14 (0.46–2.80)	0.775
Hypercholesterolemia[3]	4,215	5	0.41 (0.15–1.11)	0.079
Diabetes mellitus[3]	6,694	26	1.19 (0.47–3.02)	0.709
Gout[3]	3,265	10	0.96 (0.46–2.00)	0.907
Statin use	6,382	8	0.29 (0.12–0.68)	0.005
Metformin use	5,155	20	1.29 (0.47–3.54)	0.616
Aspirin use	5,602	19	0.70 (0.37–1.36)	0.296

[1]The p value was tested using the Wald test. [2]The prescription of hepatoprotectants was used as a surrogate marker of ALT elevation. [3]Patients with coexisting disease *vs.* patients without coexisting disease.
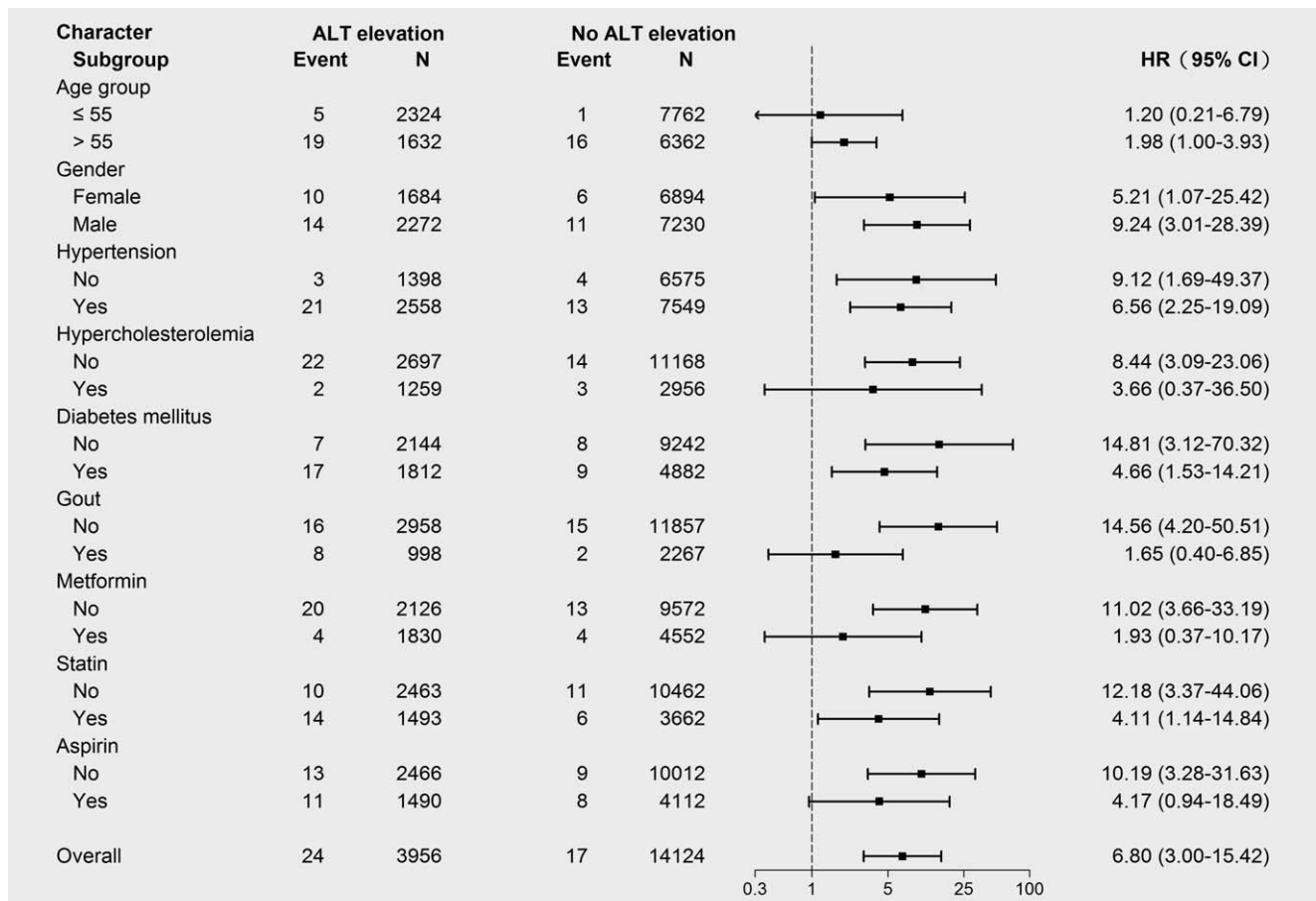

Figure 3. Multivariate stratified analyses of the association between ALT elevation and HCC development.

elevation. Additionally, cumulative incidences of HCC in various risk stratifications were calculated. The cumulative incidences of HCC in different age groups are presented in Supporting Information Figure 1. The 10-year cumulative incidences of HCC were shown to be increasing along with age levels: 18–45 years (0.19%; 95% CI, 0–0.57), 46–55 years (1.31%; 95%, CI, 0–2.86), 56–65 years (3.80%; 95% CI, 1.02–6.59) and >65 years (6.20%; 95% CI, 3.20–9.20). Furthermore, as shown in Figure 2b, the 10-year cumulative incidence of HCC was lowest in younger patients (age ≤55 years) without ALT elevation (0.36%; 95% CI, 0–1.08), followed by younger patients (age ≤55 years) with ALT elevation (1.67%; 95%, CI, 0–3.63) and finally older patients (age >55 years) without ALT elevation (2.92%; 95% CI, 1.07–4.77). Of significance, the 10-year cumulative incidence of HCC was found to be highest in older patients (age >55 years) with ALT elevation (12.41%; 95% CI, 5.99–18.83).

For further evaluating the cumulative HCC incidence after ALT elevation, we conducted an additional model where NAFLD patients with ALT elevation were followed up after the index date of hepatoprotectant prescription (Supporting Information Fig. 1). The 10-year cumulative incidence of HCC remained lowest in younger patients without ALT elevation (0.36%; 95% CI, 0–1.08), followed by younger patients with ALT elevation (1.98%; 95%, CI, 0–4.39) and finally older patients without ALT elevation (2.92%; 95% CI, 1.07–4.77). The 10-year cumulative incidence of HCC was also highest in older patients with ALT elevation (9.37%; 95% CI, 3.91–14.83).

Cumulative incidences of liver cirrhosis

Some patients may develop liver cirrhosis during their follow-up period. As shown in Supporting Information Figure 2, the cumulative incidence of cirrhosis development steadily increased, where the 10-year cumulative incidence was 2.13% (95% CI: 1.85–2.42%). In addition, as shown in Supporting Information Figure 3, the 10-year cumulative incidence of cirrhosis was lowest in younger patients without ALT elevation (1.36%; 95% CI, 0–1.08) but highest in older patients with ALT elevation (7.37%; 95% CI, 3.91–14.83).

Discussion

NAFLD-related HCC is on the increase; however, an effective strategy in HCC surveillance for noncirrhotic NAFLD patients remain waiting for development due to a high NAFLD prevalence.[6–8] Although the incidence of HCC is generally not high in noncirrhotic NAFLD patients, this long-term cohort study clearly demonstrates that the 10-year cumulative incidence of HCC could be as high as 12% in patients with an age >55 years, and an elevated serum ALT. Delayed tumor detection may contribute to both a more advanced tumor stage and decreased patient survival rates in NAFLD-related HCC.[3,25] The findings of our study suggest that HCC surveillance could be initiated for patients at an older age, with or without ALT elevation. Therefore, our

findings may provide a useful basis for prospective research in risk-oriented tumor surveillance for noncirrhotic NAFLD patients in the future.

Liver cirrhosis has been reported to be a serious risk factor for NAFLD-related HCC, where the cumulative incidence of HCC could be as high as 12.8% during a median follow-up period of 3.2 years.[5] However, our findings suggest that a proportion of patients who are initially diagnosed as clinically noncirrhotic NAFLD could be at high risk of HCC development, and using only standard follow-up procedures for cirrhotic patients would not be enough. Although current guidelines recommend regular HCC surveillance for cirrhotic patients, HCC can develop in the absence of cirrhosis in NAFLD.[5,26] In a recent prospective study of NAFLD, cirrhosis was present in only about half of HCC patients.[25] Furthermore, noncirrhotic NAFLD patients may eventually develop cirrhosis and subsequently HCC.[26]

The disease progression of NAFLD involves chronic inflammation, while HCC development requires a prolonged time frame.[2] In previous epidemiological studies, older ages were associated with a more advanced stage of NAFLD,[27] and have also been identified as an important risk factor for NAFLD-related HCC.[5] However, data on long-term follow-up for noncirrhotic NAFLD patients in different age stratifications remain sparse. This 10-year longitudinal study clearly shows that the incidence of HCC significantly increased in patients older than 55 years old, therefore, requiring special attention for these patients during HCC observation. In contrast, the incidence of HCC was found to be much lower in patients who were 55 years of age or younger, though long-term observation of many younger patients may not be either effective or practical.

ALT is traditionally considered to be an inadequate transsectional indicator representing the severity of NAFLD, particularly in cirrhotic patients.[13,14,28] However, in a recent study of biopsy-proven NAFLD, excluding advanced cirrhosis, serum ALT levels were significantly higher in patients suffering from more severe steatosis, lobular inflammation, ballooning and even fibrosis. Additionally, serum ALT could be an independent predictor of steatohepatitis.[17,18] Although serum ALT may be a prognostic marker in noncirrhotic NAFLD, a long-term follow-up study for patients with an elevated ALT is lacking. In this long-term cohort study, elevated ALT was significantly associated with an increased HCC risk, and multivariate stratified analyses also verified the association in all subgroups. Moreover, although ALT elevation may indicate the disease progression of NAFLD, the development of HCC is a long-term process. Even though the cumulative HCC incidence in younger patients with ALT elevation was quite similar to those in older patients without ALT elevation, we believe that the HCC incidences would be elevated as those in older patients with ALT elevation. In addition, we used the prescription of hepatoprotectants as a surrogate marker of ALT elevation, however, hepatoprotectants may inversely offer some protective benefits for liver

injury,[29,30] implying that the HCC risk among hepatoprotectant users could thus be underestimated. Therefore, the conclusion of our study showing that an elevated ALT could increase long-term HCC risk remains unchanged.

The potential use of several chemopreventive drugs such as statins, metformin and aspirin has been considered when attempting to lower HCC risk,[21,22,31] however, clinical evidence supporting their protective effects on NAFLD is lacking. In the present study, the use of statins was associated with a decreased HCC risk, although metformin or aspirin use was not. However, the association between drug use and various clinical conditions affecting HCC risk are quite complicated; for example, metformin users are subject to metabolic syndrome, but metabolic syndrome could in turn be related to NAFLD-related HCC.[32] Although our findings disclose some clues regarding chemoprevention, further well-controlled studies should be made in order to gain a better understanding of the protective effects of these drugs.

Several limitations should be acknowledged in our study. First, NAFLD could have been undercoded in this database, and a larger proportion of patients with an elevated ALT might thus be recruited. However, even though HCC risk in noncirrhotic NAFLD patients may have been overestimated, the incidence of HCC, particularly for patients without ALT elevation, remained low in this large cohort study. Therefore, our findings still provide a useful basis for risk-oriented tumor surveillance. Second, as in common clinical practices, most patients diagnosed with noncirrhotic NAFLD did not receive a liver biopsy, and their histopathological severity of NAFLD was not evaluated. Thus, patients suffering from subclinical liver cirrhosis could not be completely ruled out. However, even though HCC risk may have been overestimated in our study, the incidence of HCC remained low. Noninvasive parameters are therefore in demand for surveillance on a mass scale, and we used ALT elevation as a parameter. Based on our findings, a prospective study including histopathological data should be encouraged for further confirmation. Third, our database does not provide detailed laboratory data, such as serum ALT, which is the most commonly used test to evaluate liver damage. However, as previously mentioned, we used the use of hepatoprotectant as a surrogate marker for an elevated ALT according to the insurance reimbursement criteria. Although a hepatoprotectant may not have been prescribed for all patients with an elevated ALT, the HCC risk among nonhepatoprotectant users could possibly be overestimated. However, the differences in HCC risk between hepatoprotectant users and nonusers were still significant, so the conclusion of our study is not changed. Fourthly, some valuable information could not be fully obtained from the NHIRD database; for example, level of alcohol consumption.[33] Although other major causes of hepatitis, such as alcoholic liver disease and alcohol dependence, have been excluded in our study, individual information, including alcohol consumption, tobacco use, body mass index and education level, is lacking. However, even though additional factors may superimpose hepatitis on NAFLD, the findings from our study still suggest that HCC risk could be significantly increased in NAFLD patients with ALT elevation. A prospective study is certainly needed in order to provide a more complete and thorough description of the study population.

In summary, the incidence of HCC was shown to be relatively low in clinically noncirrhotic NAFLD patients, while HCC risk was found to be significantly increased in older (age >55 years) patients with an elevated serum ALT. Regular HCC surveillance may not be recommended for younger patients without ALT elevation.

Cancer Epidemiology

References

1. Zhu JZ, Dai YN, Wang YM, et al. Prevalence of nonalcoholic fatty liver disease and economy. *Dig Dis Sci* 2015;60:3194–202.
2. Satapathy SK, Sanyal AJ. Epidemiology and natural history of nonalcoholic fatty liver disease. *Semin Liver Dis* 2015;35:221–35.
3. Younossi ZM, Otgonsuren M, Henry L, et al. Association of nonalcoholic fatty liver disease (NAFLD) with hepatocellular carcinoma (HCC) in the United States from 2004 to 2009. *Hepatology* 2015;62:1723–30.
4. El-Serag HB. Epidemiology of viral hepatitis and hepatocellular carcinoma. *Gastroenterology* 2012; 142:1264–1273 e1.
5. Ascha MS, Hanouneh IA, Lopez R, et al. The incidence and risk factors of hepatocellular carcinoma in patients with nonalcoholic steatohepatitis. *Hepatology* 2010; 51:1972–8.
6. Bruix J, Sherman M, American Association for the Study of Liver D. Management of hepatocellular carcinoma: an update. *Hepatology* 2011;53: 1020–2.
7. European Association for the Study of the L, European Organisation for R, Treatment of C. EASL-EORTC clinical practice guidelines: management of hepatocellular carcinoma. *J Hepatol* 2012;56:908–43.
8. Chalasani N, Younossi Z, Lavine JE, et al. The diagnosis and management of non-alcoholic fatty liver disease: practice guideline by the American Gastroenterological Association, American Association for the Study of Liver Diseases, and American College of Gastroenterology. *Gastroenterology* 2012;142:1592–609.
9. Rafiq N, Bai C, Fang Y, et al. Long-term follow-up of patients with nonalcoholic fatty liver. *Clin Gastroenterol Hepatol* 2009;7:234–8.
10. Starley BQ, Calcagno CJ, Harrison SA. Nonalcoholic fatty liver disease and hepatocellular carcinoma: a weighty connection. *Hepatology* 2010;51: 1820–32.
11. Oda K, Uto H, Mawatari S, et al. Clinical features of hepatocellular carcinoma associated with nonalcoholic fatty liver disease: a review of human studies. *Clin J Gastroenterol* 2015;8:1–9.
12. Pearce SG, Thosani NC, Pan JJ. Noninvasive biomarkers for the diagnosis of steatohepatitis and advanced fibrosis in NAFLD. *Biomark Res* 2013;1:7.
13. Sorrentino P, Tarantino G, Conca P, et al. Silent non-alcoholic fatty liver disease-a clinical-histological study. *J Hepatol* 2004;41:751–7.
14. Browning JD, Szczepaniak LS, Dobbins R, et al. Prevalence of hepatic steatosis in an urban population in the United States: impact of ethnicity. *Hepatology* 2004;40:1387–95.

15. Miyake K, Miyake N, Kondo S, et al. Seasonal variation in liver function tests: a time-series analysis of outpatient data. *Ann Clin Biochem* 2009;46:377–84.

16. Kim SR, Nakajima T, Ando K, et al. Two cases of non-alcoholic steato-hepatitis developing from simple fatty liver. *J Gastrointestin Liver Dis* 2009; 18:491–5.

17. Verma S, Jensen D, Hart J, et al. Predictive value of ALT levels for non-alcoholic steatohepatitis (NASH) and advanced fibrosis in non-alcoholic fatty liver disease (NAFLD). *Liver Int* 2013;33: 1398–405.

18. Ballestri S, Nascimbeni F, Romagnoli D, et al. The independent predictors of NASH and its individual histological features. Insulin resistance, serum uric acid, metabolic syndrome, ALT and serum total cholesterol are a clue to pathogenesis and candidate targets for treatment. *Hepatol Res* 2016;46:1074–87.

19. Rachel Lu JF, Chiang TL. Evolution of Taiwan's health care system. *Health Econ Policy Law* 2011; 6:85–107.

20. Wu CY, Chen YJ, Ho HJ, et al. Association between nucleoside analogues and risk of hepatitis B virus-related hepatocellular carcinoma recurrence following liver resection. *JAMA* 2012;308: 1906–14.

21. Chen HP, Shieh JJ, Chang CC, et al. Metformin decreases hepatocellular carcinoma risk in a dose-dependent manner: population-based and in vitro studies. *Gut* 2013;62:606–15.

22. Wu CY, Lin JT, Ho HJ, et al. Association of nucleos(t)ide analogue therapy with reduced risk of hepatocellular carcinoma in patients with chronic hepatitis B: a nationwide cohort study. *Gastroenterology* 2014;147:143–51.

23. Lee TY, Lin JT, Ho HJ, et al. Evaluation of the effect of cumulative operator experience on hepatocellular carcinoma recurrence after primary treatment with radiofrequency ablation. *Radiology* 2015;141864.

24. Yeh CC, Lin JT, Jeng LB, et al. Non-steroidal anti-inflammatory drugs are associated with reduced risk of early hepatocellular carcinoma recurrence after curative liver resection: a nationwide cohort study. *Ann Surg* 2015;261: 521–6.

25. Piscaglia F, Svegliati-Baroni G, Barchetti A, et al. Clinical patterns of hepatocellular carcinoma in nonalcoholic fatty liver disease: a multicenter prospective study. *Hepatology* 2016;63:827–38.

26. Adams LA, Lymp JF, St Sauver J, et al. The natural history of nonalcoholic fatty liver disease: a population-based cohort study. *Gastroenterology* 2005;129:113–21.

27. Clark JM. The epidemiology of nonalcoholic fatty liver disease in adults. *J Clin Gastroenterol* 2006; 40Suppl1:S5–10.

28. Choudhary NS, Saraf N, Saigal S, et al. Estimation of normal values of serum transaminases based on liver histology in healthy Asian Indians. *J Gastroenterol Hepatol* 2015;30:763–6.

29. Aller R, Izaola O, Gomez S, et al. Effect of silymarin plus vitamin E in patients with non-alcoholic fatty liver disease. A randomized clinical pilot study. *Eur Rev Med Pharmacol Sci* 2015;19:3118–24.

30. Loguercio C, Andreone P, Brisc C, et al. Silybin combined with phosphatidylcholine and vitamin E in patients with nonalcoholic fatty liver disease: a randomized controlled trial. *Free Radic Biol Med* 2012;52:1658–65.

31. Singh S, Singh PP, Roberts LR, et al. Chemopreventive strategies in hepatocellular carcinoma. *Nat Rev Gastroenterol Hepatol* 2014;11:45–54.

32. Perumpail RB, Wong RJ, Ahmed A, et al. Hepatocellular carcinoma in the setting of non-cirrhotic nonalcoholic fatty liver disease and the metabolic syndrome: US experience. *Dig Dis Sci* 2015;60:3142–8.

33. Anstee QM, Targher G, Day CP. Progression of NAFLD to diabetes mellitus, cardiovascular disease or cirrhosis. *Nat Rev Gastroenterol Hepatol* 2013;10:330–44.

Cancer Epidemiology