Received: 2 February 2024 | First decision: 21 March 2024 | Accepted: 14 May 2024

DOI: 10.1111/apt.18063

AP&T Alimentary Pharmacology & Therapeutics WILEY

Serum growth differentiation factor 15 is a novel biomarker with high predictive capability for liver cancer occurrence in patients with MASLD regardless of liver fibrosis

Shusuke Kumazaki[1] | Hayato Hikita[1] | Yuki Tahata[1] | Ji Hyun Sung[1] | Kenji Fukumoto[1] | Yuta Myojin[1] | Sadatsugu Sakane[1] | Kazuhiro Murai[1] | Yoichi Sasaki[1] | Kumiko Shirai[1] | Yoshinobu Saito[1] | Takahiro Kodama[1] | Naruyasu Kakita[2] | Hirokazu Takahashi[3] | Hidenori Toyoda[4] | Goki Suda[5] | Eiichi Morii[6] | Takashi Kojima[7] | Takeshi Ebihara[7] | Kentaro Shimizu[7] | Yutaka Sasaki[8] | Tomohide Tatsumi[1] | Tetsuo Takehara[1]

[1]Department of Gastroenterology and Hepatology, Osaka University Graduate School of Medicine, Osaka, Japan

[2]Department of Gastroenterology and Hepatology, Kaizuka City Hospital, Osaka, Japan

[3]Liver Center, Saga University Hospital, Faculty of Medicine, Saga University, Saga, Japan

[4]Department of Gastroenterology and Hepatology, Ogaki Municipal Hospital, Ogaki, Japan

[5]Department of Gastroenterology and Hepatology, Graduate School of Medicine, Hokkaido University, Sapporo, Japan

[6]Department of Pathology, Osaka University Graduate School of Medicine, Osaka, Japan

[7]Department of Traumatology and Acute Critical Medicine, Osaka University Graduate School of Medicine, Osaka, Japan

[8]Department of Gastroenterology, Osaka Central Hospital, Osaka, Japan

Correspondence
Tetsuo Takehara, Department of Gastroenterology and Hepatology, Graduate School of Medicine, Osaka University, 2-2 Yamadaoka, Suita, Osaka 565-0871, Japan.
Email: takehara@gh.med.osaka-u.ac.jp

Funding information
Japan Agency for Medical Research and Development, Grant/Award Number: JP23fk0210121; Japan Society for the Promotion of Science, Grant/Award Number: JP23H02894

Summary

Background and Aims: Although metabolic dysfunction-associated steatotic liver disease (MASLD) patients with a Fib-4 index >1.3 are recommended for fibrosis evaluation via elastography or biopsy, a more convenient method identifying high-risk populations requiring follow-up is needed. We explored the utility of serum levels of growth differentiation factor-15 (GDF15), a cell stress-responsive cytokine related to metabolic syndrome, for stratifying the risk of clinical events in MASLD patients.

Methods: Serum GDF15 levels were measured in 518 biopsy-performed MASLD patients, 216 MASLD patients for validation, and 361 health checkup recipients with MASLD.

Results: In the biopsy-MASLD cohort, multivariate analysis indicated that the serum GDF15 level was a risk factor for liver cancer, independent of the fibrosis stage or Fib-4 index. Using a GDF15 cutoff of 1.75 ng/mL based on the Youden index, high-GDF15 patients, regardless of fibrosis status, had a higher liver cancer incidence

The Handling Editor for this article was Dr Rohit Loomba, and it was accepted for publication after full peer-eview.

rate. While patients with a Fib-4 index <1.3 or low-GDF15 rarely developed liver cancer, high-GDF15 patients with a Fib-4 index >1.3 developed liver cancer and decompensated liver events at significantly higher rates and had poorer prognoses. In the validation cohort, high-GDF15 patients had significantly higher incidences of liver cancer and decompensated liver events and poorer prognoses than low-GDF15 patients, whether limited to high-Fib-4 patients. Among health checkup recipients with MASLD, 23.0% had a Fib-4 index >1.3, 2.7% had a Fib-4 index >1.3 and >1.75 ng/mL GDF15.

Conclusions: Serum GDF15 is a biomarker for liver cancer with high predictive capability and is useful for identifying MASLD patients requiring regular surveillance.

1 | INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) is estimated to affect 25% of the worldwide population and has become the most common cause of chronic liver disease.[1] Some patients with NAFLD develop liver cancer or decompensated liver cirrhosis, which leads to poor prognosis. With an increase in the number of patients with NAFLD, the incidence of NAFLD-associated liver cancer has been increasing and is expected to increase.[2,3] Early detection of liver cancer is directly linked to improved patient prognosis,[4] and regular screening using imaging and tumour markers is important for early detection.[5] However, as the incidence of liver cancer in the NAFLD population is relatively low,[6] it would be beneficial to identify high-risk patients who require liver cancer surveillance in terms of cost-effectiveness.

The current liver cancer screening strategy depends on the liver fibrosis stage since fibrosis is a strong risk factor for the development of carcinoma (HCC).[6–8] According to the European Association for the Study of the Liver (EASL),[9] American Association for the Study of Liver Diseases (AASLD),[10] and Japanese guidelines,[11,12] patients with NAFLD with a Fib-4 index >1.3 are recommended to undergo fibrosis evaluation by elastography or liver biopsy, and patients with advanced fibrosis are recommended to undergo liver cancer surveillance. Indeed, as liver cancer occurrence in patients with a Fib-4 index <1.3 is extremely rare (approximately 0%[13]), it is reasonable to exclude these patients from liver cancer surveillance. On the other hand, in terms of cost-effectiveness, among patients with a Fib-4 index >1.3, those with advanced fibrosis are recommended to undergo liver cancer surveillance, but those without advanced fibrosis are not. However, some patients without advanced fibrosis will develop liver cancer.[6,14] Thus, the development of a novel biomarker to predict liver cancer development in patients with NAFLD, independent of fibrosis stage, is in high demand.

Growth differentiation factor 15 (GDF15) is a member of the transforming growth factor-β (TGF-β) superfamily and is a stress-responsive cytokine that reflects oxidative stress, endoplasmic reticulum stress, and inflammation.[15–19] It has been reported that serum GDF15 levels are increased in patients with inflammatory diseases, cardiovascular (CV) events, metabolic syndrome, and various cancers.[20,21] Recently, we showed that the serum GDF15 level is a predictive marker for hepatitis C virus (HCV)-associated HCC after direct-acting antiviral treatment.[22] The serum GDF15 level has been reported to be elevated in patients with hepatitis B virus-associated HCC.[23] Furthermore, GDF15 levels were shown to be elevated in patients with nonalcoholic steatohepatitis (NASH) compared to patients with NAFLD and in F3/F4 patients compared to F0-2 patients in a study of 190 patients with NAFLD.[24]

Recently, the criteria and nomenclature for steatotic liver disease have been updated.[25–27] Steatotic liver disease in conjunction with a cardiometabolic risk factor and no other cause is called metabolic dysfunction-associated steatotic liver disease (MASLD), which is the most common cause of steatotic liver disease.[25–27] Thus, the present study aimed to evaluate the utility of serum GDF15 levels as a novel predictive marker for liver-related events, including liver cancer occurrence, decompensated liver event occurrence, and prognosis, in patients with MASLD.

2 | MATERIALS AND METHODS

2.1 | Study populations

The present study is a retrospective, multicenter study. We included biopsy-proven patients with NAFLD (the biopsy cohort) without a history of liver cancer and with stored serum samples that were available at the time of analysis; these patients were from the following 4 institutions and were registered from January 2005 to May 2021: Osaka University Hospital (Suita, Japan), Kaizuka City Hospital (Kaizuka, Japan), Saga University Hospital (Saga, Japan), and Ogaki Municipal Hospital (Ogaki, Japan). We included a validation cohort of clinically diagnosed patients with NAFLD without a history of liver cancer and with stored serum samples available at the time of analysis; the patients in this cohort were from Hokkaido University Hospital (Sapporo, Japan) and were registered from August 2002 to December 2021. We excluded the following patients from the present study: (1) biopsy cohort patients with less than 5% steatosis according to a reassessment of liver histology; (2) patients diagnosed with other chronic liver diseases; (3) patients with less than 6 months of follow-up; and (4) patients diagnosed with liver cancer

within 3 months of the beginning of the observation. Finally, following the recent change in the nomenclature, patients without records of any cardiometabolic risk factor, which is the prerequisite for diagnosing MASLD,[25–27] were excluded. In the biopsy-MASLD cohort, 456/517 (88.2%) patients had a BMI ≥23, 447/518 (86.3%) patients had a fasting blood sugar (FBS) level ≥100, an HbA1c level ≥5.7 or diabetes mellitus (DM), and 0 out of the 518 patients were ultimately excluded from the biopsy cohort because of the last criterion (Figure S1). In the validation cohort, 174/200 (87.0%) patients had a BMI ≥23, 164/216 (75.9%) patients had an FBS ≥100 and an HbA1c ≥5.7 or DM, and 3 out of 219 patients were ultimately excluded from the validation cohort (Figure S2). All patients provided written informed consent, and the study design conformed to the tenets of the Declaration of Helsinki. The study protocol was approved by the Institutional Review Board (IRB) committees of Osaka University Hospital (IRB no. 17032), and permission to conduct the study was obtained from all the directors of each institution.

2.2 | Follow-up

Patients underwent liver cancer surveillance via ultrasonography and/or CT/MRI every 6–12 months. The Japanese guideline[11,12] does not deny screening for liver cancer in patients without advanced fibrosis at the present time since a certain number of patients without advanced fibrosis develop liver cancer. Indeed, in Japan, because ultrasonography is relatively easy to access, routine liver cancer screening by ultrasonography for patients without advanced fibrosis is performed as a general practice at the discretion of physicians. Liver cancer was diagnosed using typical contrast-enhanced CT imaging and/or MRI following the Japanese Society of Hepatology recommendation.[11,12] If the image was insufficient for diagnosing liver cancer, a targeted biopsy was performed, and the diagnosis was based on histological analysis. The endpoint of liver cancer occurrence was either the date of liver cancer occurrence or the date of the last imaging test for liver cancer surveillance.

2.3 | Decompensated liver events and CV events

We identified decompensated liver events and CV events that led to hospitalisation. Decompensated liver events were defined as ascites, hepatic encephalopathy, gastrointestinal bleeding due to oesophagogastric varices, and preventive treatments, including endoscopic variceal ligation and endoscopic injection sclerotherapy. CV events were defined as ischemic heart disease and stroke.

2.4 | Health checkup recipient cohort

We collected sera from 1109 individuals who underwent health checkups, including ultrasonography, at Osaka Central Hospital (Osaka, Osaka) from April 2023 to November 2023. Among them, 434 individuals were diagnosed with steatotic liver disease using ultrasonography. Individuals who were positive for HBs antigen or HCV antibody, who consumed more than 210 g of alcohol per week for males and 140 g of alcohol per week for females, and who had no record of any cardiometabolic risk factor (Table S1), which is a prerequisite for the diagnosis of MASLD,[25–27] were excluded. The serum GDF15 levels of 364 health checkup recipients who met the MASLD criteria were measured (Figure S3). All health checkup recipients provided written informed consent, and the study design conformed to the tenets of the Declaration of Helsinki. The study protocol was approved by the Institutional Review Board (IRB) committees of Osaka University Hospital (IRB no. 17032), and permission to conduct the study was obtained from all the directors of each institution.

2.5 | Serum GDF15 examination

Serum samples were collected at the start of follow-up and stored at −80°C at each institution. The serum GDF15 levels of patients with MASLD in the biopsy cohort and validation cohort were measured with an enzyme-linked immunosorbent assay (ELISA) kit for humans (#DGD150, R&D Systems, Minneapolis, MN) according to the manufacturer's protocol, as previously described.[22,28] The serum GDF15 levels of health checkup recipients who met the MASLD criteria were measured using an Elecsys GDF-15 (Roche Diagnostics, Switzerland).

2.6 | Liver histology

All liver biopsy specimens in the biopsy cohort were collected and reassessed by an experienced liver pathologist at Osaka University in June 2023. We assessed liver steatosis, inflammation, hepatocellular ballooning, and fibrosis stage using the Nonalcoholic Steatohepatitis Clinical Research Network (NASH CRN) scoring system[29] and defined stage 3 or 4 fibrosis as advanced fibrosis. We defined patients with metabolic dysfunction-associated steatohepatitis (MASH) as those with MASLD who were diagnosed with NASH by a pathologist.

2.7 | Statistical analysis

Continuous variables are presented as medians and interquartile ranges, and categorical variables are presented as real numbers for each category. Statistical analysis was performed with the Mann–Whitney *U* test to compare two continuous variables and with the chi-squared test to compare two categorical variables. Trends were examined with the Jonckheere-Terpstra trend test. To analyse the predictive power of the nomograms for liver cancer occurrence, we constructed a receiver operating characteristic (ROC) curve and examined the area under the ROC curve (AUROC). We compared the AUROC with that of the Delong test. To evaluate time-dependent predictivity,

we used a time-dependent ROC curve. For analysis of event occurrence, including liver cancer incidence, decompensated liver events, CV events, liver-related death, and overall death, we constructed Kaplan–Meier curves and followed patients until the occurrence of each event or the last day of follow-up. We applied the log-rank test to compare the incidence of events between the two groups and a Cox proportional hazards model to compare the risk. Variables for multivariate Cox regression analysis were determined by confounding factors, hazard ratios, and p values in univariate Cox regression analysis. Statistical significance was set at $p<0.05$. JMP 8.4.2 for Windows (GraphPad PRISM RRID; SCR_014242) was utilised for the analyses.

3 | RESULTS

3.1 | Serum GDF15 levels are increased in patients with MASH and increase with fibrosis

We analysed 518 patients with MASLD who underwent liver biopsy in the biopsy-MASLD cohort (Table 1). The median age was 61 years, and 59% of the patients were female. The median BMI was 27.2 kg/m^2, and 48% of the patients were diagnosed with DM. The median GDF15 level was 1.23 ng/mL (Figure 1A) and was higher in patients with MASH than in patients without MASH (Figure 1B). GDF15 levels gradually increased with the progression of fibrosis (Figure 1C). GDF15 levels were significantly higher in patients with a high Fib-4 index (>1.3) than in the other patients (Figure 1D), and the level of GDF15 was weakly correlated with the Fib-4 index (Figure 1E). However, the

TABLE 1 Patient characteristics in the biopsy-MASLD cohort.

	Missing numbers	Median (IQR)
Observation time (months)	0	64.3 (43.2–96.0)
Age (years)	0	61 (50–68)
Sex (M/F)	0	214/304
BMI	1	27.2 (24.9–30.3)
AST	0	52 (36–75)
ALT	0	67 (45–99)
GGT	0	63 (42–105)
ALP	0	254 (201–312)
T-Bil	0	0.70 (0.58–1.0)
TG	4	144 (105–204)
LDL-C	11	120 (100–143)
FBS	1	109 (98–132)
HbA1c	8	6.0 (5.5–6.7)
DM (yes/no)	0	251/267
Metformin (yes/no)	0	80/438
AFP	49	3.7 (2.6–5.3)
Alb	0	4.4 (4.1–4.6)
PT	13	97.5 (87.3–110.0)
Platelets	0	21.3 (17.1–26.0)
Fib-4 index	0	1.84 (1.06–2.81)
Non-MASH/MASH	0	234/284
Fibrosis stage (0/1/2/3/4)	0	106/160/116/84/52

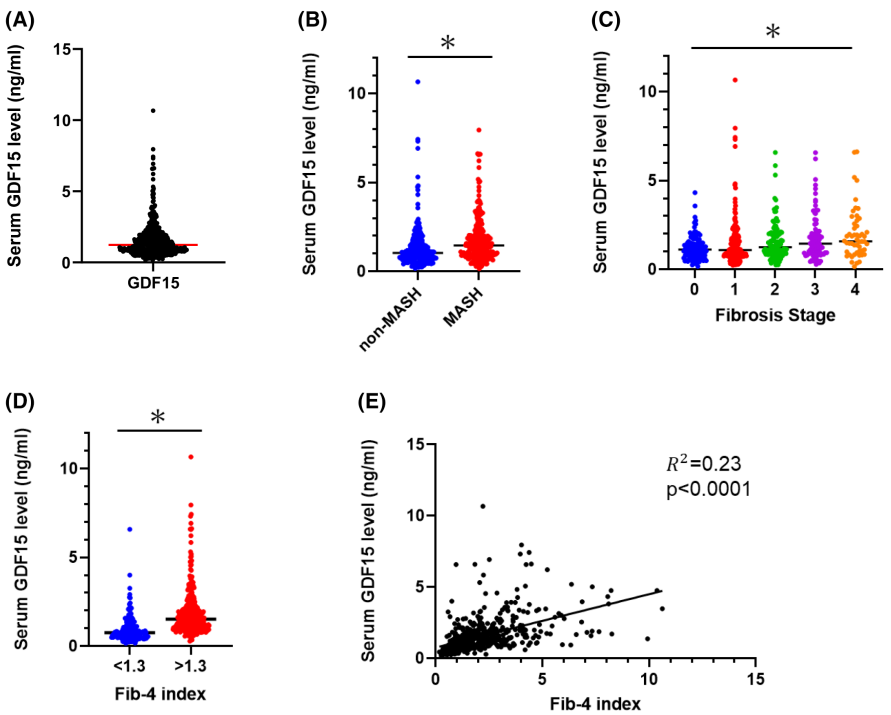

FIGURE 1 Serum GDF15 levels gradually increase with fibrosis progression. (A–D) Serum GDF15 levels in the biopsy-MASLD cohort. Distribution of serum GDF15 levels (A). Serum GDF15 levels in non-MASH ($n=234$) and MASH ($n=284$) patients (B). Statistical analysis was performed with the Mann–Whitney *U* test. Serum GDF15 levels at each fibrosis stage (C) (F0 [$n=106$], F1 [$n=160$], F2 [$n=116$], F3 [$n=84$], F4 [$n=52$]). Trends were examined with the Jonckheere–Terpstra trend test. Serum GDF15 levels in patients with a Fib-4 index <1.3 ($n=171$) or a Fib-4 index >1.3 ($n=347$) (D). The results of the statistical analysis were evaluated with the Mann–Whitney U test. The relationship between GDF15 and the Fib-4 index was assessed with the Pearson correlation coefficient (E). *$p<0.05$.

predictive ability of GDF15 in patients with advanced liver fibrosis (F3/F4) tended to be lower than that of the Fib-4 index, although the difference was not statistically significant (Figure S4A,B). When we divided all patients into GDF15-high and GDF15-low groups according to the median serum GDF15 level, patients in the GDF15-high group were older and had higher AST, γGTP, ALP, HbA1c, AFP, and Fib-4 index levels than those in the other group (Table S2).

3.2 | Both the serum GDF15 level and the Fib-4 index or liver fibrosis stage are independent factors for liver cancer occurrence

During the median follow-up of 63 months, 22 out of 518 patients developed liver cancer—21 HCCs and 1 intrahepatic cholangiocarcinoma. The incidence rates of liver cancer were 1.4% at 3 years, 2.2% at 5 years, and 4.5% at 7 years (Figure S5A). The incidence of liver cancer in patients with MASH tended to be higher than that in patients without MASH (Figure S5B). The univariate Cox proportional hazards model revealed that age, platelet count, GGT, LDL cholesterol, albumin, prothrombin time, AFP, liver fibrosis stage, Fib-4 index, and GDF15 were risk factors for liver cancer occurrence (Table 2). Considering the number of liver cancer occurrences, four multivariate Cox regression models (sets 1–4) were created to analyse risk factors for liver cancer occurrence according to the methods described in the supporting Materials and Methods section. In all models, both GDF15 and the Fib-4 index were identified as independent risk factors associated with liver cancer development (Table 2, Table S3). When selecting the liver fibrosis stage instead of the Fib-4 index, both GDF15 and liver fibrosis stage were identified as independent risk factors associated with liver cancer development (Table 3, Table S4).

3.3 | Serum GDF15 level is highly predictive of liver cancer occurrence in MASLD patients

According to the ROC curves of the serum GDF15 level for the prediction of 3-year, 5-year, and 7-year liver cancer incidence, the AUROCs were 0.949, 0.941, and 0.940, respectively. These values were greater than those of the Fib-4 index, although the difference was not significant according to the DeLong test (Figure 2A–C). Using a serum GDF15 cutoff value of 1.75, according to the Youden index of the ROC curve for 7-year incidence, 29.5% of the patients were classified into the GDF15-high subgroup. The liver cancer incidence rates at 3, 5, and 7 years in GDF15-high patients were

TABLE 2 Cox proportional hazards model for liver cancer occurrence.

	Missing numbers	Univariate analysis median (95% CI)	*p*	Multivariate analysis median (95% CI)	*p*	Multivariate analysis median (95% CI)	*p*
Age (years)	0	1.08 (1.04–1.13)	0.0003				
Sex	0	1.66 (0.72–3.94)	0.2353				
BMI (kg/m^2)	1	1.00 (0.90–1.10)	0.9755				
Platelets, ×10^4/μL	0	0.79 (0.72–0.86)	<0.0001				
AST (U/L)	0	1.01 (1.00–1.02)	0.0638				
ALT (U/L)	0	1.00 (0.98–1.00)	0.3372				
GGT (U/L)	0	1.003 (1.000–1.005)	0.0184				
ALP (U/L)	0	1.003 (0.999–1.006)	0.0956				
Total bilirubin (mg/dL)	0	1.33 (0.38–3.78)	0.6214				
Triglyceride (mg/dL)	4	0.99 (0.99–1.00)	0.1361				
LDL-cholesterol (mg/dL)	12	0.98 (0.97–1.00)	0.0191				
FBS (mg/dL)	1	1.00 (0.99–1.01)	0.8228				
HbA1c (%)	8	0.90 (0.60–1.22)	0.5758				
DM	0	0.80 (0.34–1.84)	0.5949				
Metformin	0	0.64 (0.10–2.19)	0.5428				
Albumin (g/dL)	0	0.16 (0.07–0.43)	<0.0001			0.56 (0.17–2.07)	0.3674
PT (%)	14	0.92 (0.89–0.95)	<0.0001				
AFP (ng/mL)	53	1.03 (0.99–1.05)	0.0230	1.04 (0.98–1.07)	0.0615		
GDF15 (ng/mL)	0	1.61 (1.36–1.86)	<0.0001	1.49 (1.22–1.76)	<0.0001	1.43 (1.15–1.72)	0.0004
FIB-4 Index	0	1.70 (1.45–1.98)	<0.0001	1.56 (1.29–1.85)	<0.0001	1.57 (1.29–1.88)	<0.0001
F0-2/F3-4	0	2.65 (1.15–6.13)	0.0223				

TABLE 3 Cox proportional hazards model for liver cancer occurrence.

	Missing numbers	Multivariate analysis, median (95% CI)	*p*	Multivariate analysis median (95% CI)	*p*
Albumin (g/dL)	0			0.37 (0.13–1.16)	0.0762
AFP (ng/mL)	53	1.03 (0.98–1.05)	0.0696		
GDF15 (ng/mL)	0	1.68 (1.41–1.99)	<0.0001	1.53 (1.25–1.84)	<0.0001
F0-2/F3-4	0	2.90 (1.18–7.12)	0.0200	2.71 (1.16–6.37)	0.0220

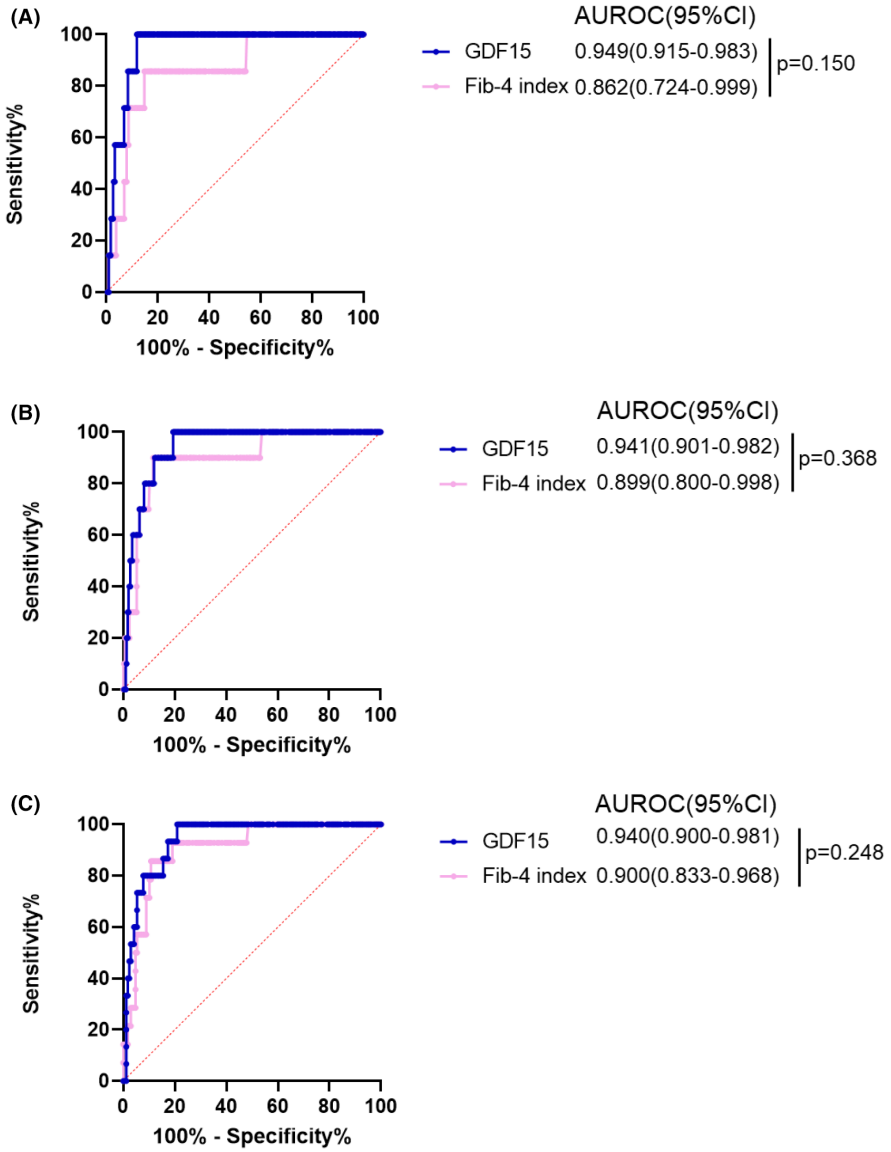

FIGURE 2 High AUROC of GDF15 for liver cancer occurrence. AUROC of GDF15 and the Fib-4 index for the prediction of liver cancer occurrence within 3, 5, and 7 years. The statistical analysis was performed with the Delong test.

4.8%, 7.7%, and 14.9%, respectively (Figure 3A). In contrast, the incidence rates in the GDF15-low patients were 0.0%, 0.0%, and 0.7%, respectively, which were significantly lower than those in the GDF15-high patients (Figure 3A). Regarding the liver fibrosis stage, the incidence rates of liver cancer in patients with advanced fibrosis (F3/F4) were 2.4%, 3.4%, and 8.3% at 3, 5, and 7 years, respectively, which were significantly higher than the incidence rates of 1.1%, 1.8%, and 3.2% at 3, 5 and 7 years, respectively, in patients without advanced fibrosis (F0/1/2) (Figure 3B).

Among patients with and without advanced fibrosis, the incidence of liver cancer in GDF15-high patients was significantly greater than that in GDF15-low patients (Figure 3C). Regarding the Fib-4 index, among patients with a Fib-4 index <1.3, none developed liver cancer within 7 years (Figure 3D). Among patients with a Fib-4 index >1.3, not only patients with advanced fibrosis (F3/4) but also a few patients with nonadvanced fibrosis (F0/1/2) developed liver cancer (Figure 3F). However, we could stratify the risk of liver cancer development using serum GDF15 levels (Figure 3E). The liver

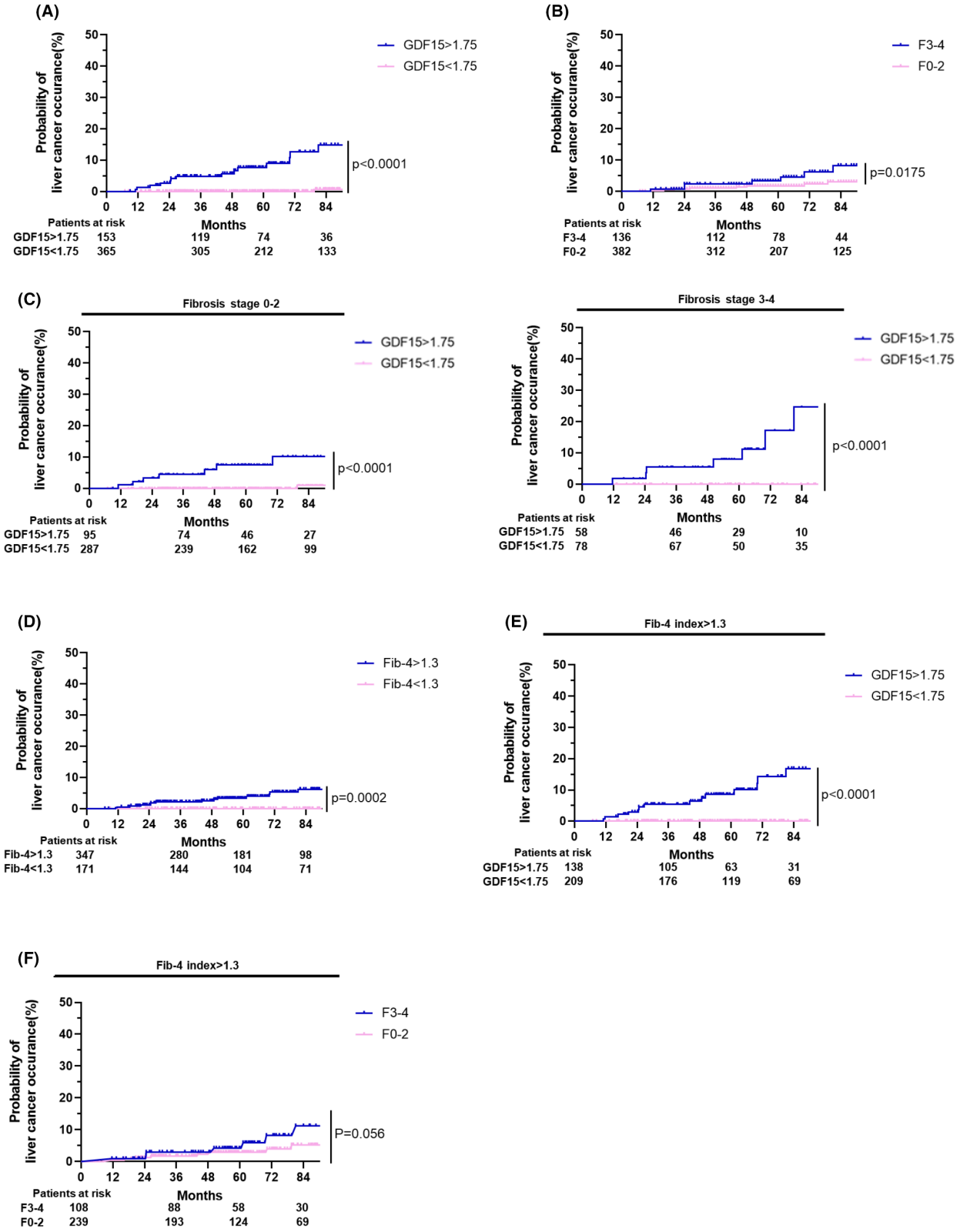

FIGURE 3 Patients with a high GDF15 level, a high Fib-4 index or advanced fibrosis are at high risk for liver cancer occurrence. (A–F) Kaplan–Meier curve for liver cancer occurrence in the biopsy-MASLD cohort ($n = 518$). All patients were divided by GDF15 level (A). All patients were divided by fibrosis status (F0/1/2 vs. F3/4) (B). Patients without advanced fibrosis (F0/1/2) or with advanced fibrosis (F3/4) divided by the GDF15 level (C). All patients were divided according to the Fib-4 index (D). Patients with a Fib-4 index >1.3 divided by the GDF15 level (E). Patients with a Fib-4 index >1.3 divided by fibrosis status (F0/1/2 vs. F3/4) (F). The statistical analysis was performed with the log-rank test.

cancer incidence rates in GDF15-high patients with a Fib-4 index >1.3 were 5.3%, 8.6%, and 16.9% at 3, 5, and 7 years, respectively, and were significantly higher than those in GDF15-low patients with a Fib-4 index >1.3, which were 0.0%, 0.0%, and 1.4% at 3, 5, and 7 years, respectively (Figure 3E).

3.4 | Patients with high serum GDF15 levels are at high risk for decompensated liver events and poor prognosis

Next, we analysed other outcomes, including decompensated liver events, CV events, and deaths. In the biopsy-MASLD cohort, 24 patients developed decompensation events, 16 developed CV events, and 12 died (7 liver-related deaths). With the same GDF15 cutoff value of 1.75 ng/mL, the incidence rate of decompensated liver events in GDF15-high patients was 7.3% at 5 years, which was significantly greater than that in GDF15-low patients, whose incidence rate of decompensated liver events was 1.4% at 5 years (Figure 4A). Patients with high GDF15 levels had significantly higher incidence rates of liver-related death and overall death (Figure 4B,C). Even when limited to patients with a Fib-4 index >1.3, patients with high GDF15 levels had significantly higher incidence rates of decompensated liver events, liver-related death, and overall death (Figure 4A–C). There was no significant difference in the incidence of CV events between GDF15-high and GDF15-low patients when the cutoff value was 1.75 ng/mL (Figure 4D). However, the difference was significant when we used a cutoff value of 3.0 ng/mL, which we set based on the Youden index (Figure 4D).

3.5 | High GDF15 levels predict high liver cancer occurrence and poor prognosis in the validation cohort

To validate the utility of GDF15 as a predictive marker for liver cancer and other events, we examined GDF15 expression in a validation cohort. The median age was 54 years, and 52% were female. The median BMI was 28.1 kg/m^2, and 38% of the patients were diagnosed with DM (Table S5). The median GDF15 level was 1.25 ng/mL (Figure 5A). During the median follow-up of 48 months, 9 out of 216 patients developed liver cancer, and all of them were HCC. The incidence rates of liver cancer were 2.9% at 3 years, 6.2% at 5 years, and 7.7% at 7 years (Figure 5B). Using the GDF15 cutoff value of 1.75 ng/mL, 72 patients (33.3%) were classified as GDF15-high patients. The incidence rates of liver cancer in these patients were 7.2%, 16.8%, and 20.6% at 3, 5, and 7 years, respectively, and were significantly higher than those in patients with low GDF15 levels, whose incidence rate of liver cancer was 0.8% from 3 to 7 years (Figure 5C). Among patients with a Fib-4 index <1.3, the incidence rate of liver cancer was low, at 1.5% from 3 to 7 years (Figure 5B). On the other hand, among patients with a Fib-4 index >1.3, the incidence rates of liver cancer in patients with high GDF15 levels were 6.1%, 16.5%, and 20.7% at 3, 5, and 7 years, respectively, and were significantly higher than those in patients with low GDF15 levels, whose incidence rate of liver cancer was 1.8% from 3 to 7 years (Figure 5C). In the validation cohort, 14 patients developed decompensated liver events, and seven patients died (five liver-related deaths). All of these patients had a Fib-4 index >1.3. According to the GDF15 cutoff value of 1.75 ng/mL, patients with high GDF15 levels were at a significantly higher risk of decompensated liver events, liver-related death, and overall death, whether limited to patients with a Fib-4 index >1.3 or not (Figure 5D–F). The combination of a Fib-4 index >1.3 and a GDF15 > 1.75 ng/mL narrowed the number of MASLD patients in the validation cohort to 29.2% (63/216), and this combination could be an efficient marker for identifying patients at high risk for liver carcinogenesis or other related events.

3.6 | Among those who underwent medical checkups and met the MASLD criteria, 2.8% had a Fib4 index >1.3 and a GDF15 > 1.75 ng/mL

Next, we analysed how the criteria for a Fib-4 index >1.3 and a GDF15 > 1.75 ng/mL can narrow down MASLD patients in health checkup recipients. The serum GDF15 level was measured in 364 individuals who met the MASLD criteria among health checkup recipients for whom stocked sera were available. The median age was 52 years, and the median Fib4 index was 0.94 (Table S6). The median serum GDF15 level was 0.76 ng/dL. Among the 364 individuals, 18 (4.9%) had a GDF15 > 1.75 ng/mL, 86 (23.6%) had a Fib4 index >1.3, and 10 (2.7%) had both (Figure S6).

4 | DISCUSSION

In the present study, we found that the serum GDF15 level is a novel biomarker for predicting liver cancer occurrence and has a high predictive capability in patients with MASLD. Among patients with MASLD, liver fibrosis is a well-known risk factor for liver cancer occurrence.[7,8] Consistent with these previous reports, the incidence rates in patients with MASLD with advanced fibrosis (F3/F4) were significantly higher than those in patients with MASLD with nonadvanced fibrosis (F0/1/2) (Figure 3B). Importantly, even among patients with advanced fibrosis (F3/F4), the risk of liver cancer occurrence could be stratified by the serum GDF15 level. Among patients with advanced fibrosis, the 3-, 5-, and 7-year cumulative liver carcinogenesis rates of patients with a GDF15 > 1.75 ng/mL were 5.4%, 7.9%, and 24.7%, respectively, while none of the patients with a GDF15 ≤ 1.75 ng/m developed liver cancer within 7 years (Figure 3C). On the other hand, the 3-, 5-, and 7-year incidence rates in patients with MASLD with nonadvanced fibrosis (F0/1/2) were 1.1%, 1.8%, and 3.2%, respectively (Figure 3B). Consistent with previous reports,[6,14] liver

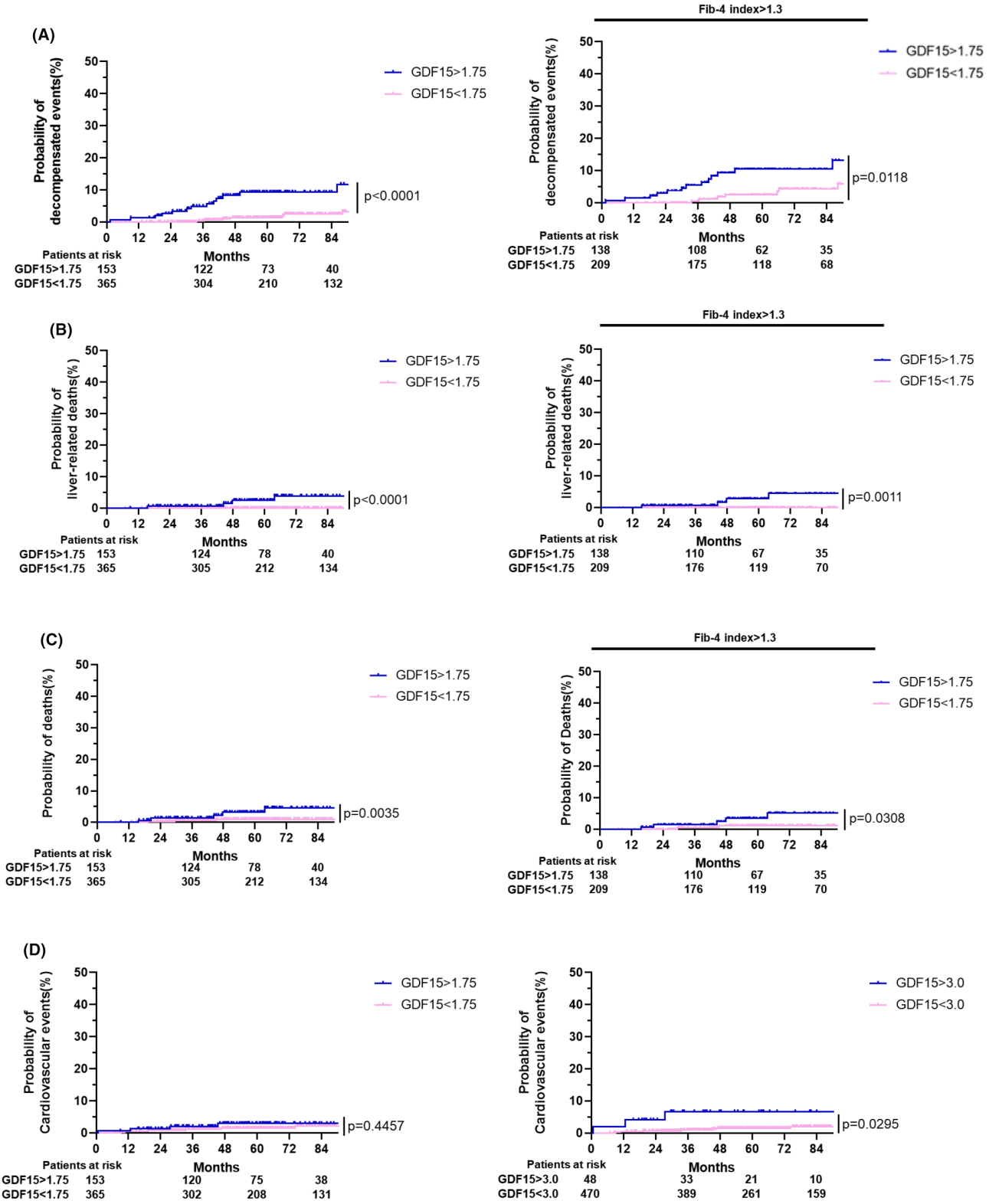

FIGURE 4 Patients with high GDF15 levels are at high risk for decompensated liver events and poor prognosis. (A–C) Kaplan–Meier curves for decompensated liver events (A), liver-related deaths (B), and overall deaths (C) in all patients ($n = 518$) or patients with a Fib-4 index >1.3 ($n = 347$) in the biopsy-MASLD cohort divided by the GDF15 level. (D) Kaplan–Meier curves for cardiovascular events in all patients in the biopsy-MASLD cohort ($n = 518$) divided by GDF15 level. The statistical analysis was performed with the log-rank test.

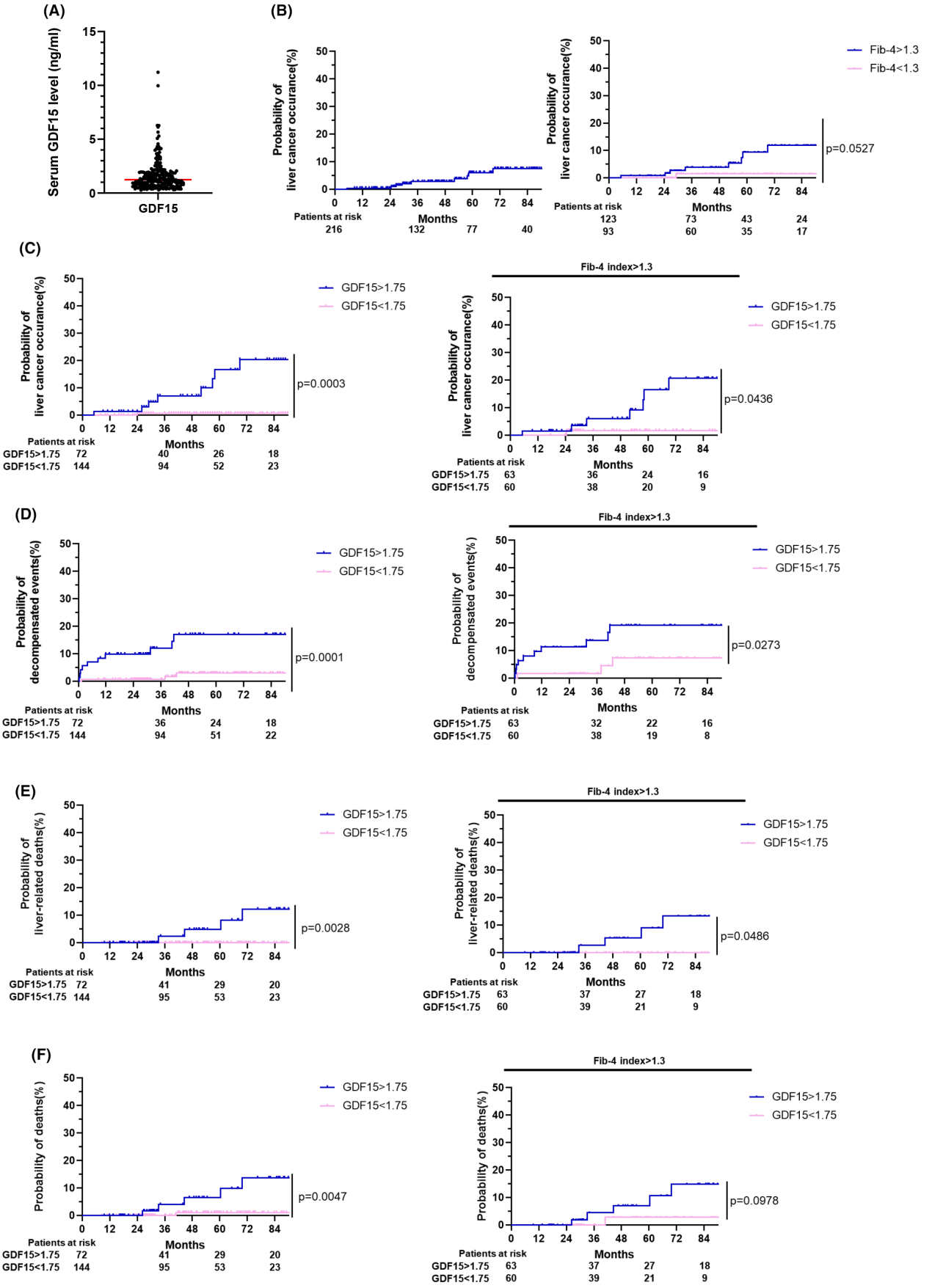

FIGURE 5 NAFLD patients with high GDF15 levels are at high risk for liver cancer, decompensated liver events, liver-related death and overall death. (A) Distribution of serum GDF15 levels in the validation cohort ($n=216$). (B) Kaplan–Meier curve for liver cancer occurrence in the validation cohort ($n=216$). All patients and all patients were divided by the Fib-4 index. (C–F) Kaplan–Meier curves for liver cancer occurrence (C), decompensated liver events (D), liver-related deaths (E), and overall deaths (F) in all patients ($n=216$) or patients with a Fib-4 index >1.3 ($n=123$) in the validation cohort divided by the GDF15 level. The statistical analysis was performed with the log-rank test.

cancer also develops in patients with MASLD with nonadvanced fibrosis (F0/1/2), which is problematic when considering carcinogenesis follow-up targets for patients with MASLD. The present study showed that GDF15 could also clearly stratify the risk of liver cancer occurrence in patients with nonadvanced fibrosis (F0/1/2) (Figure 3C). In the present study, we revealed that high serum GDF15 is a novel risk factor for liver cancer occurrence regardless of liver fibrosis status.

The guidelines recommend the evaluation of liver fibrosis in patients with MASLD with a Fib-4 index >1.3 by elastography or liver biopsy and follow-up according to the degree of fibrosis.[9–12] As previously reported,[13] the incidence of liver cancer in patients with MASLD with a Fib-4 index <1.3 was quite low (Figures 3D and 5B), supporting the exclusion of these patients from liver cancer surveillance. The present study clearly showed that while patients with MASLD with a Fib-4 index <1.3 or GDF15 < 1.75 ng/m rarely developed liver cancer (Figures 3A,D and 5B,C), patients with MASLD with a Fib-4 index >1.3 and GDF15 > 1.75 ng/mL developed liver cancer at a high frequency (Figures 3E and 5C). Among 347 patients with MASLD with a Fib-4 index >1.3 in the biopsy-MASLD cohort, 239 patients who were diagnosed with nonadvanced fibrosis (F0/1/2) by liver biopsy developed liver cancer (3.0% at 5 years; Figure 3F), while none of the 208 patients with GDF15 < 1.75 ng/mL developed liver cancer within 5 years (Figure 3E). Patients with MASLD with a Fib-4 index >1.3 and GDF15 > 1.75 ng/mL also had a significantly greater risk of decompensated liver events and poor prognosis (Figures 4A–C and 5D–F). Based on our results, patients with MASLD with a Fib-4 index >1.3 and GDF15 > 1.75 ng/mL should be followed up regularly, regardless of the degree of liver fibrosis.

Among the 518 (biopsy-MASLD cohort) and 216 (validation cohort) hospital patients with MASLD, 67.0% and 56.9%, respectively, had a Fib-4 index >1.3, and 26.6% and 29.2%, respectively, had a Fib-4 index >1.3 and GDF15 > 1.75 ng/mL. On the other hand, among the 361 health checkup recipients who met the MASLD criteria, 23.6% had a Fib4 index >1.3, and only 2.7% had a Fib-4 index >1.3 and a GDF15 > 1.75 ng/mL. If the results of the present study on hospital outpatients with MASLD regarding clinical outcomes can be adapted to health checkup recipients, the combination of the Fib-4 index and GDF15 would be able to pick up high-risk patients efficiently from patients with MASLD and could be a useful biomarker in clinical settings, not only at liver specialty clinics or hospitals but also at primary care clinics or health checks.

In previous studies, serum GDF15 levels were shown to be beneficial for predicting the prognosis of acute coronary syndrome and heart failure patients.[30–32] One study reported that serum GDF15 levels are related to the risk of CV events, such as carotid artery plaques and reduced left ventricular ejection fraction, in community-dwelling individuals.[33] In the present study, patients with MASLD with a GDF15 level >1.75 ng/dL were not at high risk for CV events, but patients with MASLD with a GDF15 level >3.0 ng/dL had a significantly greater risk of developing CV events. In the validation cohort, CV events occurred in only 2 patients, and both had a GDF15 level >3.0 ng/dL. Nevertheless, the event number was too small to determine whether patients with MASLD with high GDF15 levels are at high risk for CV events in the present study; thus, further study is necessary.

In the present study, the serum GDF15 level was identified as a risk factor for liver cancer occurrence independent of liver fibrosis, suggesting that GDF15 is also a factor other than liver fibrosis, although it was correlated to some extent with fibrosis (Figure 1C). Increased levels of GDF15 have been reported in response to oxidative stress,[34,35] which is one of the critical factors in the process of liver carcinogenesis in patients with chronic hepatitis, namely, viral hepatitis[36] and NAFLD.[37,38] Indeed, continuous hepatocyte apoptosis, a characteristic feature of chronic liver disease, promotes liver cancer occurrence and progression via oxidative stress.[39–41] Thus, GDF15 is associated with oxidative stress, which is closely related to liver cancer occurrence and progression.

In conclusion, the serum GDF15 level is a novel biomarker for liver cancer occurrence, decompensated liver events, and death in patients with MASLD. The serum GDF15 level combined with the Fib-4 index is beneficial for identifying high-risk patients with MASLD who need regular surveillance. We propose measuring serum GDF15 levels in MASLD patients with a Fib-4 index>1.3, and if the serum GDF15 level is greater than 1.75 ng/mL, we suggest performing regular liver cancer screening regardless of the fibrosis stage. This approach is expected to efficiently detect cases of liver carcinogenesis not only in advanced fibrosis patients but also in nonadvanced fibrosis patients, and it is expected to lead to the early detection of liver cancer in larger numbers of MASLD patients.

AUTHOR CONTRIBUTIONS

Shusuke Kumazaki: Conceptualization; methodology; data curation; visualization; project administration; writing – original draft; writing – review and editing; formal analysis; software; investigation; validation. **Hayato Hikita:** Conceptualization; methodology; writing – review and editing; writing – original draft; data curation; formal analysis; funding acquisition; investigation; validation. **Yuki Tahata:** Writing – review and editing. **Ji Hyun Sung:** Writing – review and editing. **Kenji Fukumoto:** Writing – review and editing. **Yuta Myojin:** Conceptualization; methodology; writing – review and editing. **Sadatsugu Sakane:** Writing – review and editing. **Kazuhiro Murai:** Writing – review and editing. **Yoichi Sasaki:** Writing – review and editing. **Kumiko Shirai:** Writing – review and editing. **Yoshinobu**

Saito: Writing – review and editing. **Takahiro Kodama:** Writing – review and editing. **Naruyasu Kakita:** Writing – review and editing. **Hirokazu Takahashi:** Writing – review and editing. **Hidenori Toyoda:** Writing – review and editing. **Goki Suda:** Writing – review and editing. **Eiichi Morii:** Data curation; writing – review and editing. **Takashi Kojima:** Writing – review and editing. **Takeshi Ebihara:** Writing – review and editing. **Kentaro Shimizu:** Writing – review and editing. **Yutaka Sasaki:** Writing – review and editing. **Tomohide Tatsumi:** Writing – review and editing. **Tetsuo Takehara:** Conceptualization; methodology; writing – review and editing; funding acquisition; supervision.

ACKNOWLEDGEMENTS

We would like to express our deepest gratitude to Dr. Riichiro Nezu, Dr. Takeyoshi Yumiba, and Dr. Akira Amemiya (Osaka Central Hospital) for supporting the collection of the clinical samples and data and Dr. Rina Okada (Department of Gastroenterology and Hepatology, Osaka University Graduate School of Medicine) for collecting the clinical data.

Declaration of personal interests: The authors have no conflicts of interest relevant to the content to declare.

FUNDING INFORMATION

This study was partly supported by a Grant-in-Aid for Research from the Japan Agency for Medical Research and Development (JP23fk0210121 to T. Takehara) and a Grant-in-Aid for Scientific Research (JP23H02894 to H.H.) from the Japan Society for the Promotion of Science, Japan.

AUTHORSHIP

Guarantor of the article: Tetsuo Takehara.

ORCID

Hidenori Toyoda https://orcid.org/0000-0002-1652-6168
Goki Suda https://orcid.org/0000-0003-0098-9106
Tetsuo Takehara https://orcid.org/0000-0001-5036-3457

REFERENCES

1. Riazi K, Azhari H, Charette JH, Underwood FE, King JA, Afshar EE, et al. The prevalence and incidence of NAFLD worldwide: a systematic review and meta-analysis. Lancet Gastroenterol Hepatol. 2022;7(9):851–61. https://doi.org/10.1016/S2468-1253(22)00165-0
2. Liu C, Zhu S, Zhang J, Wu P, Wang X, du S, et al. Global, regional, and national burden of liver cancer due to non-alcoholic steatohepatitis, 1990-2019: a decomposition and age-period-cohort analysis. J Gastroenterol. 2023;58(12):1222–36. https://doi.org/10.1007/s00535-023-02040-4
3. Estes C, Anstee QM, Arias-Loste MT, Bantel H, Bellentani S, Caballeria J, et al. Modeling NAFLD disease burden in China, France, Germany, Italy, Japan, Spain, United Kingdom, and United States for the period 2016-2030. J Hepatol. 2018;69(4):896–904. https://doi.org/10.1016/j.jhep.2018.05.036
4. Singal AG, Pillai A, Tiro J. Early detection, curative treatment, and survival rates for hepatocellular carcinoma surveillance in patients with cirrhosis: a meta-analysis. PLoS Med. 2014;11(4):e1001624. https://doi.org/10.1371/journal.pmed.1001624
5. Onyirioha K, Mittal SG, Singal A. Is hepatocellular carcinoma surveillance in high-risk populations effective? Hepat Oncol. 2020;7(3):HEP25. https://doi.org/10.2217/hep-2020-0012
6. Kanwal F, Kramer JR, Mapakshi S, Natarajan Y, Chayanupatkul M, Richardson PA, et al. Risk of hepatocellular cancer in patients with non-alcoholic fatty liver disease. Gastroenterology. 2018;155(6):1828–1837.e2. https://doi.org/10.1053/j.gastro.2018.08.024
7. Ekstedt M, Hagström H, Nasr P, Fredrikson M, Stål P, Kechagias S, et al. Fibrosis stage is the strongest predictor for disease-specific mortality in NAFLD after up to 33 years of follow-up. Hepatology. 2015;61(5):1547–54. https://doi.org/10.1002/hep.27368
8. Chayama K, Hiramatsu A, Shima T, Itoh Y, Yamaguchi K, Nakajima T, et al. Impact of fibrosis on liver-related event incidence in non-alcoholic fatty liver disease: a multicenter observational study. Hepatol Res. 2023;53(12):1169–84. https://doi.org/10.1111/hepr.13950
9. easloffice@easloffice.eu EAftSotLEa, Panel CPG, Chair:, representative: EGB, members: P. EASL clinical practice guidelines on non-invasive tests for evaluation of liver disease severity and prognosis - 2021 update. J Hepatol. 2021;75(3):659–89. https://doi.org/10.1016/j.jhep.2021.05.025
10. Rinella ME, Neuschwander-Tetri BA, Siddiqui MS, Abdelmalek MF, Caldwell S, Barb D, et al. AASLD Practice Guidance on the clinical assessment and management of nonalcoholic fatty liver disease. Hepatology. 2023;77(5):1797–835. https://doi.org/10.1097/HEP.0000000000000323
11. Tokushige K, Ikejima K, Ono M, Eguchi Y, Kamada Y, Itoh Y, et al. Evidence-based clinical practice guidelines for nonalcoholic fatty liver disease/nonalcoholic steatohepatitis 2020. J Gastroenterol. 2021;56(11):951–63. https://doi.org/10.1007/s00535-021-01796-x
12. Tokushige K, Ikejima K, Ono M, Eguchi Y, Kamada Y, Itoh Y, et al. Evidence-based clinical practice guidelines for nonalcoholic fatty liver disease/nonalcoholic steatohepatitis 2020. Hepatol Res. 2021;51(10):1013–25. https://doi.org/10.1111/hepr.13688
13. Loosen SH, Kostev K, Keitel V, Tacke F, Roderburg C, Luedde T. An elevated FIB-4 score predicts liver cancer development: a longitudinal analysis from 29,999 patients with NAFLD. J Hepatol. 2022;76(1):247–8. https://doi.org/10.1016/j.jhep.2021.08.030
14. Fujii H, Iwaki M, Hayashi H, Toyoda H, Oeda S, Hyogo H, et al. Clinical outcomes in biopsy-proven nonalcoholic fatty liver disease patients: a multicenter registry-based cohort study. Clin Gastroenterol Hepatol. 2023;21(2):370–9. https://doi.org/10.1016/j.cgh.2022.01.002
15. Wang D, Day EA, Townsend LK, Djordjevic D, Jørgensen SB, Steinberg GR. GDF15: emerging biology and therapeutic applications for obesity and cardiometabolic disease. Nat Rev Endocrinol. 2021;17(10):592–607. https://doi.org/10.1038/s41574-021-00529-7
16. Breit SN, Brown DA, Tsai VW. The GDF15-GFRAL pathway in health and metabolic disease: friend or foe? Annu Rev Physiol. 2021;83:127–51. https://doi.org/10.1146/annurev-physiol-022020-045449
17. Keipert S, Ost M. Stress-induced FGF21 and GDF15 in obesity and obesity resistance. Trends Endocrinol Metab. 2021;32(11):904–15. https://doi.org/10.1016/j.tem.2021.08.008
18. Johann K, Kleinert M, Klaus S. The Role of GDF15 as a Myomitokine. Cells. 2021;10(11):2990. https://doi.org/10.3390/cells10112990
19. Lockhart SM, Saudek V, O'Rahilly S. GDF15: a hormone conveying somatic distress to the brain. Endocr Rev. 2020;41(4):bnaa007. https://doi.org/10.1210/endrev/bnaa007
20. Wallentin L, Hijazi Z, Andersson U, Alexander JH, de Caterina R, Hanna M, et al. Growth differentiation factor 15, a marker of oxidative stress and inflammation, for risk assessment in patients with atrial fibrillation: insights from the Apixaban for Reduction in Stroke and Other Thromboembolic Events in Atrial Fibrillation

(ARISTOTLE) trial. Circulation. 2014;130(21):1847–58. https://doi.org/10.1161/CIRCULATIONAHA.114.011204
21. Asrih M, Wei S, Nguyen TT, Yi HS, Ryu D, Gariani K. Overview of growth differentiation factor 15 in metabolic syndrome. J Cell Mol Med. 2023;27(9):1157–67. https://doi.org/10.1111/jcmm.17725
22. Myojin Y, Hikita H, Tahata Y, Doi A, Kato S, Sasaki Y, et al. Serum growth differentiation factor 15 predicts hepatocellular carcinoma occurrence after hepatitis C virus elimination. Aliment Pharmacol Ther. 2022;55(4):422–33. https://doi.org/10.1111/apt.16691
23. Chen J, Tang D, Xu C, Niu Z, Li H, Li Y, et al. Evaluation of serum GDF15, AFP, and PIVKA-II as diagnostic markers for HBV-associated hepatocellular carcinoma. Lab Med. 2021;52(4):381–9. https://doi.org/10.1093/labmed/lmaa089
24. Koo BK, Um SH, Seo DS, Joo SK, Bae JM, Park JH, et al. Growth differentiation factor 15 predicts advanced fibrosis in biopsy-proven non-alcoholic fatty liver disease. Liver Int. 2018;38(4):695–705. https://doi.org/10.1111/liv.13587
25. Rinella ME, Lazarus JV, Ratziu V, Francque SM, Sanyal AJ, Kanwal F, et al. A multisociety Delphi consensus statement on new fatty liver disease nomenclature. J Hepatol. 2023;79(6):1542–56. https://doi.org/10.1016/j.jhep.2023.06.003
26. Rinella ME, Lazarus JV, Ratziu V, Francque SM, Sanyal AJ, Kanwal F, et al. A multisociety Delphi consensus statement on new fatty liver disease nomenclature. Hepatology. 2023;78(6):1966–86. https://doi.org/10.1097/HEP.0000000000000520
27. Rinella ME, Lazarus JV, Ratziu V, Francque SM, Sanyal AJ, Kanwal F, et al. A multisociety Delphi consensus statement on new fatty liver disease nomenclature. Ann Hepatol. 2023;29(1):101133. https://doi.org/10.1016/j.aohep.2023.101133
28. Myojin Y, Hikita H, Sugiyama M, Sasaki Y, Fukumoto K, Sakane S, et al. Hepatic stellate cells in hepatocellular carcinoma promote tumor growth via growth differentiation factor 15 production. Gastroenterology. 2021;160(5):1741–1754.e16. https://doi.org/10.1053/j.gastro.2020.12.015
29. Kleiner DE, Brunt EM, Van Natta M, Behling C, Contos MJ, Cummings OW, et al. Design and validation of a histological scoring system for nonalcoholic fatty liver disease. Hepatology. 2005;41(6):1313–21. https://doi.org/10.1002/hep.20701
30. Kempf T, Björklund E, Olofsson S, Lindahl B, Allhoff T, Peter T, et al. Growth-differentiation factor-15 improves risk stratification in ST-segment elevation myocardial infarction. Eur Heart J. 2007;28(23):2858–65. https://doi.org/10.1093/eurheartj/ehm465
31. Wallentin L, Lindhagen L, Ärnström E, Husted S, Janzon M, Johnsen SP, et al. Early invasive versus non-invasive treatment in patients with non-ST-elevation acute coronary syndrome (FRISC-II): 15 year follow-up of a prospective, randomised, multicentre study. Lancet. 2016;388(10054):1903–11. https://doi.org/10.1016/S0140-6736(16)31276-4
32. Wollert KC, Kempf T. Growth differentiation factor 15 in heart failure: an update. Curr Heart Fail Rep. 2012;9(4):337–45. https://doi.org/10.1007/s11897-012-0113-9
33. Lind L, Wallentin L, Kempf T, Tapken H, Quint A, Lindahl B, et al. Growth-differentiation factor-15 is an independent marker of cardiovascular dysfunction and disease in the elderly: results from the Prospective Investigation of the Vasculature in Uppsala Seniors (PIVUS) Study. Eur Heart J. 2009;30(19):2346–53. https://doi.org/10.1093/eurheartj/ehp261
34. Tsai VWW, Husaini Y, Sainsbury A, Brown DA, Breit SN. The MIC-1/GDF15-GFRAL pathway in energy homeostasis: implications for obesity, cachexia, and other associated diseases. Cell Metab. 2018;28(3):353–68. https://doi.org/10.1016/j.cmet.2018.07.018
35. Kim J, Kim SH, Kang H, Lee S, Park SY, Cho Y, et al. TFEB-GDF15 axis protects against obesity and insulin resistance as a lysosomal stress response. Nat Metab. 2021;3(3):410–27. https://doi.org/10.1038/s42255-021-00368-w
36. Ivanov AV, Valuev-Elliston VT, Tyurina DA, Ivanova ON, Kochetkov SN, Bartosch B, et al. Oxidative stress, a trigger of hepatitis C and B virus-induced liver carcinogenesis. Oncotarget. 2017;8(3):3895–932. https://doi.org/10.18632/oncotarget.13904
37. Gabbia D, Cannella L, De Martin S. The Role of Oxidative Stress in NAFLD-NASH-HCC Transition-Focus on NADPH Oxidases. Biomedicines. 2021;9(6):687. https://doi.org/10.3390/biomedicines9060687
38. Hamaguchi K, Miyanishi K, Osuga T, Tanaka S, Ito R, Sakamoto H, et al. Association between hepatic oxidative stress related factors and activation of Wnt/β-catenin signaling in NAFLD-induced hepatocellular carcinoma. Cancers (Basel). 2022;14(9):2066. https://doi.org/10.3390/cancers14092066
39. Hikita H, Kodama T, Shimizu S, Li W, Shigekawa M, Tanaka S, et al. Bak deficiency inhibits liver carcinogenesis: a causal link between apoptosis and carcinogenesis. J Hepatol. 2012;57(1):92–100. https://doi.org/10.1016/j.jhep.2012.01.027
40. Hikita H, Kodama T, Tanaka S, Saito Y, Nozaki Y, Nakabori T, et al. Activation of the mitochondrial apoptotic pathway produces reactive oxygen species and oxidative damage in hepatocytes that contribute to liver tumorigenesis. Cancer Prev Res (Phila). 2015;8(8):693–701. https://doi.org/10.1158/1940-6207.CAPR-15-0022-T
41. Nozaki Y, Hikita H, Tanaka S, Fukumoto K, Urabe M, Sato K, et al. Persistent hepatocyte apoptosis promotes tumorigenesis from diethylnitrosamine-transformed hepatocytes through increased oxidative stress, independent of compensatory liver regeneration. Sci Rep. 2021;11(1):3363. https://doi.org/10.1038/s41598-021-83082-7

SUPPORTING INFORMATION

Additional supporting information will be found online in the Supporting Information section.

How to cite this article: Kumazaki S, Hikita H, Tahata Y, Sung JH, Fukumoto K, Myojin Y, et al. Serum growth differentiation factor 15 is a novel biomarker with high predictive capability for liver cancer occurrence in patients with MASLD regardless of liver fibrosis. Aliment Pharmacol Ther. 2024;00:1–13. https://doi.org/10.1111/apt.18063