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ORIGINAL ARTICLE

Impact of a new liver immune status index among patients with hepatocellular carcinoma after initial hepatectomy

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Abstract

Aim: The anti-tumor effects of natural killer (NK) cells vary among individuals. Tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) expressed on liver NK cells is a marker of anti-tumor cytotoxicity against hepatocellular carcinoma (HCC) in immune cell therapy. This study aimed to develop a liver immune status index (LISI) that predicts low TRAIL expression and validates its ability to predict recurrence after initial hepatectomy for primary HCC.

Methods: A functional analysis of liver NK cells co-cultured with interleukin-2 for 3 days was performed of 40 liver transplant donors. The LISI, which predicted low TRAIL expression (25% quartile: <33%) in liver NK cells, was calculated using multiple logistic regression analysis. Next, 586 initial hepatectomy cases were analyzed based on the LISI.

Results: Our model was based on the Fibrosis-4 index$^{+0.1}$ (odds ratio [OR], 1.33), body mass index (OR, 0.61), and albumin levels$^{+0.1}$ (OR, 0.54). The area under the receiver operating characteristic curve (AUC) of the LISI for low TRAIL expression was 0.89. Stratification of the recurrence rates (RR) revealed that LISI was an independent predictive factor of RR (moderate risk: hazard ratio, 1.44; high risk: hazard ratio, 3.02). The AUC was similar for the LISI, albumin–indocyanine green evaluation grade, albumin–bilirubin score, and geriatric nutritional risk index for predicting RR. Among the vascular invasion cases, the LISI was more useful than the other indexes.

Conclusion: Our model facilitates the prediction of RR in high-risk patients by providing LISI to predict the anti-tumor effects of NK cells.

KEYWORDS
anti-tumor activity, hepatectomy, hepatocellular carcinoma, natural killer cells

1 | INTRODUCTION

Hepatocellular carcinoma (HCC) is the third leading cause of cancer-related death worldwide.[1] HCC is associated with a high recurrence rate despite curative resection. Liver fibrosis is a high-risk factor for multicentric carcinogenesis.[2] HCC causes cancer-related death worldwide,[1] even after 2014, when the risk of hepatitis type C (HCV) was significantly higher in the dehydroabietic acid era. Vascular invasion and blood loss during surgery are reported risk factors for the extrahepatic recurrence of HCC.[3,4] Circulating tumor cells (CTCs) are cancer cells that are shed from a primary or metastatic tumor and then circulate in the peripheral blood, especially in cases of vascular invasion.[5]

Natural killer (NK) cells are the first layer of immune defense against infections and cancers such as intraoperative tumor spills. In particular, NK cells act as innate lymphocytes in the detection and elimination of CTC to prevent recurrences.[6] Activation receptors on NK cells, such as DNAX accessory molecule-1 (DNAM-1 or CD226), NKp46, NKp44, NKp30, and NK group 2D (NKG2D), recognize tumor cell-expressing stress-inducible ligands such as MHC class I polypeptide-related sequences A and B (MICA and MICB).[7] NK cells can cytolyze tumor cells through the perforin (PFN), granzyme B (GrB), and interferon-gamma (IFN-γ) exocytosis pathways.[8] Moreover, they display tumor necrosis factor (TNF) superfamily members, such as the Fas cell surface death receptor ligand and TNF-related apoptosis-inducing ligand (TRAIL), which trigger apoptosis in target cells upon binding to FAS (or CD95) and TRAIL-R1/R2, respectively.[9] Unlike peripheral blood NK cells, liver NK cells are immature and possess unique ligands, such as TRAIL. Liver NK cells express TRAIL and exert strong cytotoxicity against hepatoma cells via the TRAIL–TRAIL receptor pathway.[10–12] TRAIL expression in liver NK cells has been used as an antitumor marker in several clinical trials.[13–15]

However, the expression of TRAIL in liver NK cells is subject to significant individual variations. Homogeneous genotypes of TRAIL single-nucleotide polymorphisms were independent predictors of extrahepatic recurrence after hepatectomy for HCC.[16] In addition, the population of liver NK cells with weak TRAIL expression remains unknown. After hepatectomy and portal hypertension, TRAIL expression in liver NK cells decreases and promotes tumor recurrence.[12,17,18] The non-invasive prediction of liver NK cell function in HCC patients is meaningful for prognostic analysis among patients with liver disease and immunotherapy using NK cells. Hence, this study aimed to clarify the characteristics of patients with lower TRAIL expression and create a new immune index that can predict the antitumor activity of liver NK cells.

2 | MATERIALS AND METHODS

2.1 | Isolation of lymphocytes from liver graft perfusate

The donor hepatectomy was performed as described previously.[13] Thereafter, the liver graft was perfused ex vivo through the portal vein. In addition, liver mononuclear cells (LMNC) were obtained by ex vivo perfusion through the portal vein of resected livers from colorectal cancer with liver metastasis (CRLM) patients as well.[19] These samples were additionally collected as subanalysis of a randomized clinical trial in Hiroshima Surgical Study Group of Clinical Oncology (HiSCO) registered with the National Review Board (HiSC-01, University Hospital Medical Information [UMIN] 00000378) to investigate the effect of chemotherapy on the functions of intrahepatic immune cells in resectable CRLM patients.[19] LMNCs were isolated by gradient centrifugation using Ficoll-Paque (GE Healthcare Bio-Sciences, Little Chalfont, UK). LMNCs were cultured with human recombinant IL-2 (100 Japanese reference units/mL; Takeda, Japan) in complete medium at 37°C in a 5% CO_2 incubator for 3 days. One day before the infusion, 1 μg/mL of human anti-CD3 monoclonal antibody (Miltenyi Biotec) was added to opsonize the $CD3^+$ fraction as described in a previous study.[13] Low TRAIL expression (25% quartile: <33%) was observed in liver NK cells (Figure 1A).

2.2 | Flow cytometric analysis

All analyses were performed using a FACS Canto II Cytometer (BD Biosciences, Mountain View, CA, USA). To detect the surface phenotype, the leukocytes were stained with monoclonal antibodies against CD3 (clone HIT3a; BD Biosciences, Pharmingen), TRAIL (clone RIK-2; eBioscience), and CD56 (clone B159; BD Biosciences, Pharmingen). The data were analyzed using FlowJo software (Tree Star, Inc. Ashland, OR, USA). NK cells were defined as CD3− CD56+ cells as described previously.[14,15]

2.3 | Patients

The initial hepatectomy for primary HCC was performed in a single-institution study in 2009–2018. The patients' clinical demographic data at the time of hepatectomy were obtained from the electronic records. The rates of HCC recurrence and long-term survival after surgery were also obtained from the clinical records. Patients were followed up using ultrasonography, contrast-enhanced computed tomography (CT), or magnetic resonance imaging combined with an evaluation of serum α-fetoprotein (AFP) and des-γ-carboxy protein (DCP) levels at 3-month intervals for up to 3 years and 6-month intervals for up to 5 years. The diagnosis was histologically confirmed when necessary.

2.4 | Assessment of markers

The FIB-4 index, initially developed for patients with human immunodeficiency virus and hepatitis C coinfection, was calculated using the following formula[20]:

(age [years] × aspartate aminotransferase [U/L]/ (platelet count [10^9/L] × √alanine aminotransferase [U/L])). The albumin–bilirubin (ALBI) grade consists of grades 1–3 calculated using the following formula: $\log_{10}$ bilirubin (μmol/L) × 0.66 + albumin (g/L) × −0.085.[21]

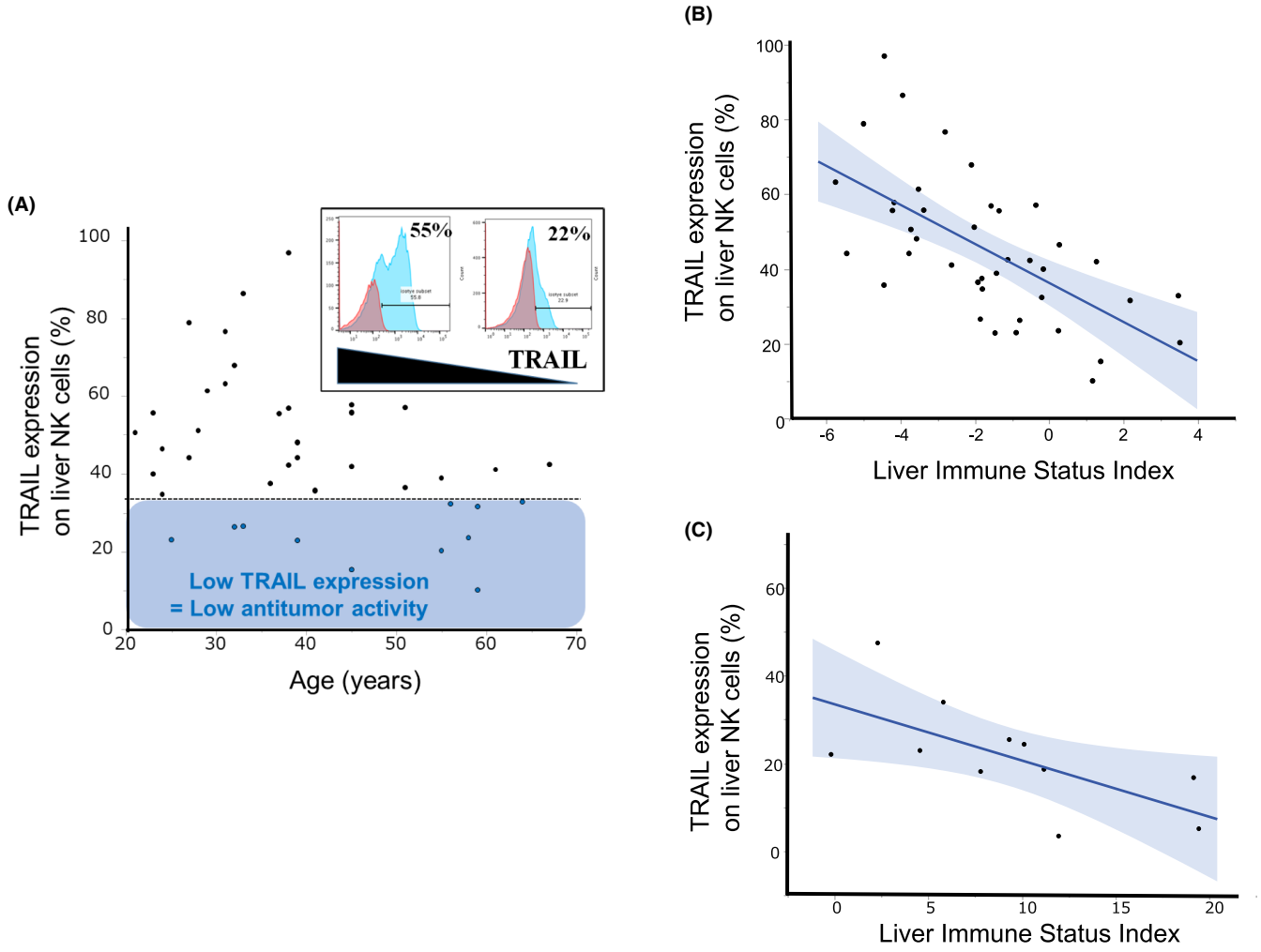

FIGURE 1 Development of a new liver immune status index. (A) The expression of liver NK cells differs individually. (B) The expression values of TRAIL on liver NK cells correlate inversely with the LISI of the living donor. (C) The expression values of TRAIL on liver NK cells correlate inversely with the LISI of colorectal cancer liver metastases.

The Albumin-IndoCyanine green Evaluation (ALICE) score was calculated, including the indocyanine green retention rate at 15 min (ICG R15) and albumin using the following formula: $0.663 \times \log_{10}$ ICG R15 (%) − $0.0718 \times$ albumin (g/L) and divided into three grades by the ALICE grade.[22]

The Geriatric Nutritional Risk Index (GNRI) formula was as follows.[23,24] GNRI = $1.489 \times$ albumin (g/L) + $41.7 \times$ present/ideal body weight (PBW/IBW) = $1.489 \times$ albumin (g/L) + $41.7 \times$ body mass index (BMI)/22. Serum albumin levels and PBW were measured upon admission and before surgery.

2.5 | Statistical analysis

The nonparametric Mann–Whitney *U*-test was performed to compare differences between the two independent groups; values of $p < 0.05$ were considered statistically significant. Values are expressed as median [min–max]. The correlation (Spearman's correlation coefficient) between the expression in liver NK cells and the liver immune status index (LISI) was analyzed. Multivariate logistic regression analyses were conducted to assess variables that were independently associated with reduced TRAIL expression in liver NK cells (defined as <33% in the 25th quartile). All variables were included in the multivariate models, and the backward elimination method, with a removal criterion of $p = 0.05$, was employed to identify the covariates.

Overall survival (OS) and recurrence rate (RR) were plotted using Kaplan–Meier analysis and compared using log-rank statistics. Multivariate analyses of variables independently related to OS and RR were performed using the Cox proportional hazards model. Uni- and multivariate Cox regression analyses were performed to assess the association of OS and RR with the following variables: sex, age, Child–Pugh classification, hepatitis virus, LISI, total bilirubin levels, prothrombin time, indocyanine green clearance test, operation time, blood loss, DCP level, serum AFP level, number of tumors, tumor size, and vascular invasion. All variables were included in the multivariate models, and the backward elimination method with a removal criterion of $p = 0.10$ was used to select the covariates. The performance

of the prognostic systems was evaluated in terms of the area under the receiver operating characteristic curve (AUROC), homogeneity, discriminatory ability, and Akaike information criterion. The AUROC was compared between each model using DeLong's method.

JMP statistical software (JMP® 14; SAS Institute Inc., Cary, NC, USA) was used for all statistical analyses.

3 | RESULTS

3.1 | Background of living donor and new immune status index

The backgrounds of the living donors are summarized in Table 1. This study included 40 living liver donors: 30 men (75.0%) and 10 women (30.0%). The median donor age and Fib-4 index were 38 years [21–67] and 0.70 [0.29–1.91], respectively. The expression of TRAIL in liver NK cells was 42.3% [9.9–97%] as determined by flow cytometry after IL-2 stimulation. The expression of TRAIL had strong individual differences (25% quartile: <33%), and TRAIL expression on liver NK cells was calculated using multiple logistic regression analysis (Figure 1A).

In the multivariate analyses, albumin levels$^{+0.1g/dL}$ (odds ratio [OR], 0.54; 95% confidence interval [CI], 0.31–0.93; $p<0.01$), Fib-4 index$^{+0.1}$ (OR, 1.33; 95% CI, 1.02–1.76; $p=0.02$), and BMI (OR, 0.61; 95% CI, 0.36–0.99; $p=0.01$) were detected as independent predictive factors of lower TRAIL expression on liver NK cells (Table 2; Figure S1). A new liver immune status index (LISI) was calculated using the following formula: 36.39 − (6.18×ALB) − (0.50×BMI)+(2.91×Fib-4 index) (Table 2). The AUROC of the LISI for low TRAIL expression was 0.89. Our results demonstrated that the expression values of TRAIL on liver NK cells inversely correlated with the LISI of living donors (Spearman correlation coefficient: −0.66; $p<0.01$; Figure 1B).

TABLE 1 Patients' characteristics among living donor hepatectomy.

Variables	N = 40
Age, years	38 [21–67]
Male	30 (75.0)
Smoking, yes	12 (30.0)
Drinking, yes	9 (22.5)
Right lobe, yes	17 (42.5)
BMI, kg/m^2	22.0 [16.2–29.4]
Fib-4 index	0.70 [0.29–1.91]
Operation time, min	448 [344–582]
Blood loss, mL	500 [146–1223]
A blood test before operation	
White blood cells	5435 [1398–14000]
Platelet, ×10^4/mL	23.6 [12.9–34.5]
Total-bilirubin, mg/dL	0.8 [0.5–2.0]
AST, IU/L	18.5 [12–39]
ALT, IU/L	19.5 [10–44]
ALP, IU/L	202 [120–328]
Albumin, g/dL	4.7 [4.2–5.3]
CRP, mg/dL	0.03 [0.02–0.29]
PT-INR, %	1.0 [0.88–1.12]
ICG-15 min, %	7.1 [2.3–15.7]
Total cholesterol, mg/dL	200 [27–355]
ChE, IU/L	345 [230–492]
TRAIL expression on liver NK cells, %	42.3 (9.9–97)

Abbreviations: AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP, Alkaline Phosphatase; ChE, cholinesterase; CRP, C reactive protein; PT-INR, prothrombin time international normalized ratio; ICGR15, indocyanine green retention rate at 15 min; TRAIL, TNF-related apoptosis-inducing ligand.

3.1.1 | Correlation between new LISI and CRLM patients

This study included 11 CRLM patients. The median Fib-4 index, BMI, and albumin levels were 3.18 [2.14–4.78], 23.4 [19.2–26.0], and 4.1 g/dL [3.9–4.5], respectively. The median LISI was 9.3 [4.5–11.9] and the median proportion of TRAIL expression on liver NK cells was 22.1% [16.8–25.5] after IL-2 stimulation. The strong inverse relationship between the percentage of TRAIL-positive NK cells and LISI in 11 patients with CRLM which included relatively moderate to severe liver fibrosis was also confirmed (Spearman correlation coefficient: −0.69; $p=0.02$, Figure 1C).

3.2 | Correlation between new LISI and clinical characteristics

This study included 586 initial hepatectomies in 453 (77.3%) men and 133 (22.7%) women. The median patient age was 71 years [31–91] and the median total tumor size was 24 mm [6–200]. Preoperative liver function was classified as Child–Pugh class A in 546 (93.2%) patients. The thresholds for high- and moderate-risk categories in HCC liver resection patients were established using quartile values of LISI distribution, specifically the 25th and 75th percentiles. The patients were divided into three liver fibrosis grades (high-risk, >15.33 [$n=146$]; moderate-risk, 4.55–15.33 [$n=294$]; and low-risk, <4.55 [$n=146$]). The distribution of these characteristics is presented in Table 3. Although the high-risk group had lower AFP levels and a smaller tumor size, the liver function parameters were significantly worse, and the proportion of HCV positive patients was higher (Table 3).

3.3 | Overall patient outcomes

The three LISI grades also stratified the OS and risk of RR. The survival rates at 1, 3, and 5 years were 96.0%, 90.2%, and 87.3% in the low-risk group; 95.0%, 82.8%, and 68.1% in the moderate-risk

group; and 88.9%, 68.9%, and 59.4% in the high-risk group, respectively ($p<0.01$; Figure 2A). The 1-, 3-, and 5-year RR were 13.3%, 30.7%, and 44.1% in the low-risk group; 20.9%, 45.9%, and 54.6% in the moderate-risk group; and 31.6%, 67.7%, and 74.8% in the high-risk group ($p<0.01$; Figure 2B).

3.4 | Factors associated with OS and recurrence

In the multivariate analysis, Child–Pugh score A (hazard ratio [HR], 0.57; 95% CI, 0.33–0.97; $p=0.04$), higher AFP levels (HR, 1.92; 95% CI, 1.33–2.77; $p<0.01$), larger tumor size (HR, 1.72; 95% CI, 1.18–2.52; $p<0.01$), vascular invasion (HR, 1.98; 95% CI, 1.31–2.98; $p<0.01$), and LISI (moderate-risk: HR, 1.98; 95% CI, 1.13–3.47; $p=0.02$; high-risk: HR, 2.84; 95% CI, 1.55–5.22; $p<0.01$) were independent predictive factors of OS. Table 4 shows the risk factors for OS. In the multivariate analysis, male sex (HR, 1.57; 95% CI, 1.11–2.22; $p=0.91$), higher DCP levels (HR, 1.38; 95% CI, 1.04–1.83; $p=0.03$), larger bleeding volume (HR, 1.35; 95% CI, 1.02–1.75; $p=0.04$), multiple tumors (HR, 1.85; 95% CI, 1.42–2.42; $p<0.01$), vascular invasion (HR, 1.80; 95% CI, 1.32–2.46; $p<0.01$), and LISI (moderate-risk: HR, 1.44; 95% CI, 1.01–2.05; $p=0.04$; high-risk: HR, 3.02; 95% CI, 2.05–4.44; $p<0.01$) were independent predictive factors of RR (Table 5).

TABLE 2 A new liver immune status index.

Predictive factor	OR	95% CI	*p*-value
Albumin $^{+0.1}$(g/dL)	0.54	0.31–0.93	<0.01
Fib-4 index $^{+0.1}$	1.33	1.02–1.76	0.02
BMI (kg/m^2)	0.61	0.36–0.99	0.01

Note: Liver Immune Status Index (LISI).Formula: 36.39 − (6.18 × Albumin) − (0.50 × BMI) + (2.91 × Fib-4 index*).

*Fib-4 index: Age × AST / Platelet ×√AST.

Abbreviation: AST, aspartate aminotransferase; ALT, alanine aminotransferase; BMI, body mass index; Fib-4 index, Fibrosis-4 index.

3.5 | Comparison of model suitability

The AUROC for 5-year OS in the LISI, ALBI, ALICE grades, Fib-4 index, and GNRI groups were 0.66, 0.70, 0.69, 0.61, and 0.70, respectively. The AUROC was similar between LISI and the other grades, whereas that for the 5-year OS for the Fib-4 index was significantly lower than that for the LISI (Figure 3A). Among the vascular invasion cases with a high risk of CTC and recurrence, the AUROC for the 5-year OS in the LISI, ALBI, ALICE grades, Fib-4 index, and GNRI were 0.73, 0.67, 0.66, 0.69, and 0.67, respectively (Figure 3B). There were no differences among these indexes. The AUROC for the 5-year RR of the LISI, ALBI, and ALICE grades, Fib-4 index, and GNRI were 0.61, 0.59, 0.60, 0.60, and 0.59, respectively. The AUROC for ISI and other grades were similar

TABLE 3 Background data according to immune status index levels.

	High risk (15.33 <)	Moderate risk (4.55–15.33)	Low risk (<4.55)	*p*-value
Subject	*N* = 146	*N* = 294	*N* = 146	
Gender Male	126 (86.3)	239 (81.3)	88 (60.3)	<0.01
Age (years)	75 [34–89]	68 [37–91]	65 [31–88]	<0.01
BMI (kg/m^2)	22.1 [15.8–33.6]	22.7 [15.3–37.3]	24.3 [16.5–37.4]	<0.01
Hepatitis type B	10 (6.9)	47 (16.0)	46 (31.5)	<0.01
Hepatitis type C	98 (67.1)	147 (50.0)	57 (39.0)	<0.01
Liver immune status index	20.75 [15.4–57.2]	9.0 [4.57–15.30]	2.2 [−7.26–4.52]	
Total-bilirubin (mg/dL)	0.8 [0.3–3.0]	0.7 [0.2–2.7]	0.9 [0.4–2.9]	<0.01
Albumin (g/dL)	3.6 [2.3–4.8]	4.0 [2.5–4.9]	4.5 [3.4–5.4]	<0.01
PT (%)	93 [33–141]	87 [24–125]	81 [21–145]	<0.01
ICGR15 (%)	21.5 [3.5–63]	12.6 [2.2–79]	10.9 [2.2–76]	<0.01
Child-Pugh Score A	119 (81.5)	282 (95.9)	145 (99.3)	<0.01
AFP (ng/mL)	5.8 [0.5–290 700]	7.0 [0.5–253 300]	16.4 [1.5–23 780]	<0.01
DCP	49 [10–54 832]	46 [1–223 940]	34 [2.8–838 500]	0.20
Operation time (min)	282 [116–769]	324 [82–695]	322 [109–460]	0.01
Bleeding volume (mL)	328 [10–7798]	360 [5–4470]	256 [5–2570]	0.04
Timor size (mm)	20 [8–200]	25 [8–200]	25 [6–150]	0.02
Multiple tumors	53 (36.3)	102 (34.7)	36 (24.7)	0.06
Vascular invasion	30 (20.7)	72 (24.5)	24 (16.4)	0.15

Abbreviations: AFP, α-fetoprotein; BMI, Body mass index; DCP, des-γ-carboxy protein; ICGR15, indocyanine green retention rate at 15 min; PT, prothrombin time.

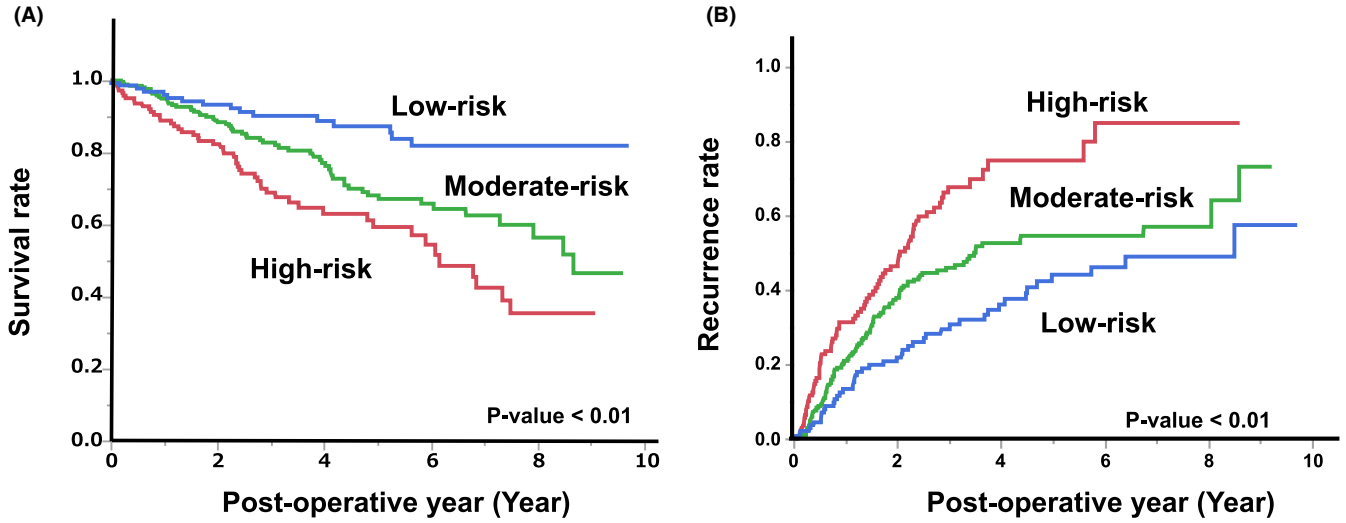

FIGURE 2 Outcomes among initial hepatectomy with primary HCC. (A) Overall survival. (B) Recurrence.

TABLE 4 Risk factors for survival.

	Univariate			Multivariate		
Factors	HR	95% CI	*p*-value	HR	95% CI	*p*-value
Age > 70 (years)	1.20	0.85–1.71	0.29			
Sex: Male	0.92	0.61–1.40	0.71			
HBV antigen positive	0.70	0.43–1.14	0.15			
HCV antibody positive	1.02	0.72–1.44	0.90			
Total bilirubin>1.5 (mg/dL)	1.67	0.84–3.29	0.14			
PT <80 (%)	1.36	0.92–1.99	0.12			
ICGR15 > 10 (%)	1.99	1.30–3.02	<0.01	1.53	0.98–2.39	0.06
Child-Pugh Score A	0.37	0.22–0.60	<0.01	0.57	0.33–0.97	0.04
AFP >40 (mAU/mL)	2.39	1.78–3.40	<0.01	1.92	1.33–2.77	<0.01
DCP >40 (ng/mL)	1.80	1.25–2.59	<0.01			
Operation time > 300 (min)	1.51	1.06–2.15	0.02			
Bleeding volume > 500 (mL)	1.93	1.36–2.75	<0.01	1.38	0.95–2.02	0.09
Tumor size>30 (mm)	1.97	1.39–2.78	<0.01	1.72	1.18–2.52	<0.01
Multiple tumors	1.84	1.30–2.61	<0.01	1.43	0.99–2.06	0.05
Vascular invasion	2.82	1.96–4.07	<0.01	1.98	1.31–2.98	<0.01
Liver immune status index						
Low risk (4.5<)	1 (reference)			1 (reference)		
Moderate risk (4.5–15.3)	2.32	1.34–4.03	<0.01	1.98	1.13–3.47	0.02
High risk (< 15.3)	3.86	2.20–6.77	<0.01	2.84	1.55–5.22	<0.01

Abbreviations: AFP, α-fetoprotein; AST, aspartate aminotransferase; DCP, des-γ-carboxy protein; ICGR15, indocyanine green retention rate at 15 min; PT, prothrombin time.

(Figure 3C). However, among the vascular invasion cases with a high risk of CTC and recurrence, the AUROC for the 5-year RR in the LISI, ALBI, ALICE grades, Fib-4 index, and GNRI were 0.64, 0.52, 0.54, 0.65, and 0.55, respectively (Figure 3D). These results suggested that the predictive power of the LISI was superior to that of the indexes for HCC cases with vascular invasion, which is a high risk of recurrence.

4 | DISCUSSION

This study revealed that the new predictive index, calculated from BMI, albumin levels, and Fib-4 index, could predict lower TRAIL expression in liver NK cells and clarified the usefulness of predicting the risk of RR, especially during the initial hepatectomy for primary HCC with vascular invasion. Compared with other liver function

TABLE 5 Risk factors for recurrence.

	Univariate			Multivariate		
Factors	HR	95% CI	*p*-value	HR	95% CI	*p*-value
Age > 70 (years)	0.95	0.74–1.23	0.72			
Sex: Male	1.34	0.97–1.89	0.08	1.57	1.11–2.22	0.01
HBV antigen positive	0.89	0.64–1.25	0.50			
HCV antibody positive	1.03	0.80–1.34	0.79			
Total bilirubin>1.5 (mg/dL)	1.27	0.74–2.19	0.38			
PT <80 (%)	1.39	1.05–1.84	0.02			
ICGR15 > 10 (%)	1.62	1.20–2.16	<0.01			
Child-Pugh Score A	0.59	0.38–0.93	0.02			
AFP >40 (mAU/mL)	1.45	1.10–1.91	<0.01			
DCP >40 (ng/mL)	1.61	1.24–2.09	<0.01	1.38	1.04–1.83	0.03
Operation time > 300 (min)	1.32	1.02–1.71	0.03			
Bleeding volume > 500 (mL)	1.84	1.41–2.39	<0.01	1.35	1.02–1.78	0.04
Tumor size>30 (mm)	1.44	1.11–1.87	<0.01			
Multiple tumors	2.08	1.61–2.70	<0.01	1.85	1.42–2.42	<0.01
Vascular invasion	2.04	1.52–2.74	<0.01	1.80	1.32–2.46	<0.01
Liver Immune status index						
Low risk (4.5<)	1 (reference)			1 (reference)		
Moderate risk (4.5–15.3)	1.54	1.09–2.18	<0.01	1.44	1.01–2.05	0.04
High risk (<15.3)	2.63	1.82–3.80	<0.01	3.02	2.05–4.44	<0.01

Abbreviations: AFP, α-fetoprotein; DCP, des-γ-carboxy protein; ICGR15, indocyanine green retention rate at 15 min; PT, prothrombin time.

indexes, including BMI and serum albumin levels, the LISI can predict RR in high-risk patients with vascular invasion. This supports the hypothesis that NK cells attack CTCs. The expression of TRAIL in NK cells exhibits individual differences. However, it is difficult to analyze the patient characteristics of TRAIL-expressing NK cells without invasive methods such as liver biopsy. Therefore, we developed a technique to collect more liver NK cells from the perfusion drainage of living liver grafts[13,14] and analyze their characteristics, allowing us to thoroughly evaluate their cytotoxic potential. This non-invasive prediction of liver NK cell function in HCC patients can play an important role in prognostic analyses in other HCC patients as well as in immunotherapy using liver NK cells.

NK cells play a crucial role in the prevention of viruses, infections, and tumors via the innate immune response.[25] NK cells play a role in the detection and elimination of CTC to prevent their recurrence.[6] The mechanisms of NK cell cytotoxicity against tumor cells are known, such as DNAM-1, NKp46, NKp44, NKp30, NKG2D, PFN, GrB, and IFN-γ.[7] In terms of intrahepatic immunity, immature NK cells are found in higher proportions in the liver, producing higher cytokine and TRAIL levels.[26–28] Liver NK cells, which comprise a large proportion of immature NK cells, express TRAIL and exert strong cytotoxicity through the TRAIL–TRAIL receptor pathway.[10–13] TRAIL expression in liver NK cells has been used as a cytotoxicity marker in several clinical trials of liver transplantation.[13–15] Since this score is derived from a comprehensive hematological index, it has the potential to reflect not only the immune status within the liver but also the systemic immune status to some extent. Although previous studies have investigated the activity of peripheral blood NK cells in patients with HCC,[29] the current report is distinctive in that it focuses specifically on liver NK cells. TRAIL-expressing liver NK cells can function as key players in preventing CTC and their recurrence, and there are many individual differences in TRAIL expression in liver NK cells. The significance of this research lies in the fact that the LISI score, which is an accurate predictor of TRAIL expression specifically in liver NK cells with interindividual variability, represents a valuable parameter for stratifying high-risk patients with vascular invasion following hepatic resection. Our results indicate that TRAIL expression in liver NK cells was inversely correlated with the LISI among donor graft and CRLM patients. If the usefulness of this index becomes clear, it could be applied not only to liver resection of HCC but also to cases of unresectable HCC using immune checkpoint inhibitors, liver resection for CRLM, and cholangiocarcinoma.

Several studies demonstrated a relationship between Fib-4 index and long-term outcomes in HCC patients after hepatectomy.[30,31] The LISI included not only the Fib-4 index but also albumin levels; therefore, the AUROC for 5-year OS for the LISI was higher than that for the Fib-4 index (0.66 vs. 0.61, $p < 0.01$). In cases with vascular invasion, the AUROC for the 5-year OS of LISI was high at 0.73. The GNRI, calculated from BMI and serum albumin levels, focused on evaluating patients with many malignant tumors,[32,33] and a lower GNRI value is reportedly associated with

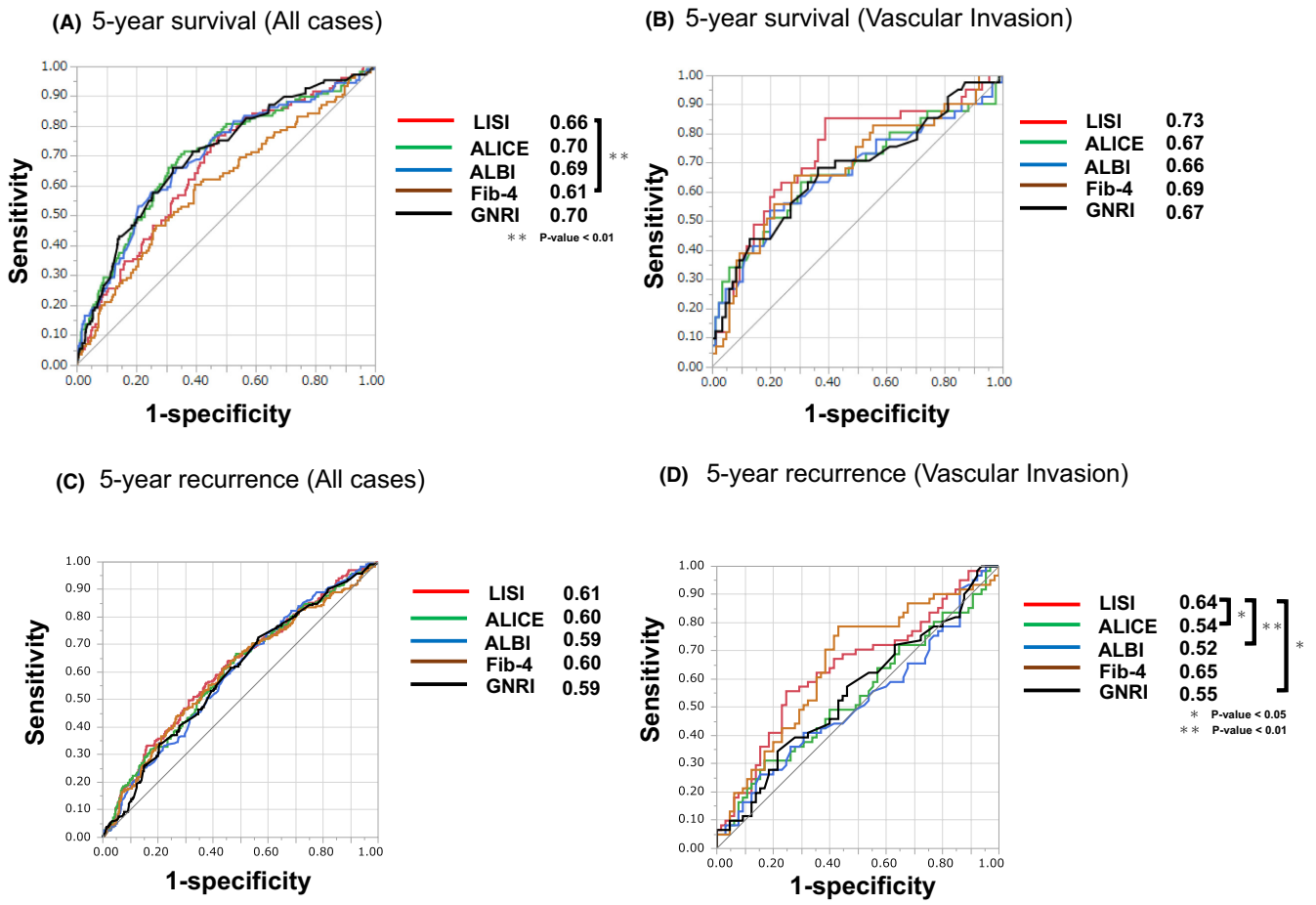

FIGURE 3 The area under the curves between ISI and other grades for predicting 5-year survival and recurrence. (A) 5-year survival in all cases. (B) 5-year survival in cases with vascular invasion. (C) 5-year recurrence in all cases. (D) 5-year recurrence in cases with vascular invasion.

poor prognosis after hepatectomy in elderly HCC patients.[24,34] Our results showed that liver NK cell function was strongly correlated with liver fibrosis, BMI, and nutritional status. These results support those of a previous study that reported that cirrhotic recipients in need of liver transplantation had lower TRAIL expression from liver NK cells.[11] Besides, a high BMI was positively correlated with TRAIL expression in liver NK cells. However, all Japanese patients were enrolled in this study. Therefore, the BMI was 16.2%–29.4%, relatively lower than that of other races. It is necessary to determine the effect of a higher BMI (>30%) on liver NK cells in other populations.

RR could be stratified by LISI grade, an independent predictive factor of RR (moderate-risk: HR, 1.44; high-risk: HR, 3.02). The AUC of LISI, ALICE grade, ALBI score, and GNRI for predicting RR were similar. Among the vascular invasion cases, the LISI was more useful than the other indexes. This result supports the role of NK cells in the detection and elimination of CTC, particularly in cases with a high risk of recurrence and vascular invasion. The ALBI score, which is based solely on serum albumin and bilirubin levels, is an objective parameter of liver functional reserve in HCC and has been validated by several independent research groups.[21,35] ALICE grade, which uses albumin levels and ICG R15, has also been reported as a precise preoperative evaluator of liver function.[22] Interestingly, compared with other liver function indexes, including BMI and serum albumin levels, this new index could predict RR in high-risk patients with vascular invasion. These results support the hypothesis that NK cells attack CTCs and prevent their recurrence. Intrahepatic metastasis is thought to occur frequently in HCC with vascular invasion, wherein microtumors invade the blood circulation, and liver NK cells are believed to play a critical role in preventing such metastasis. Conversely, multicentric recurrence is strongly influenced by the background liver conditions, such as viral hepatitis or a highly fibrotic liver. Since the LISI incorporates the Fib-4 index, it was also probable to have a relation with multicentric recurrence. Nevertheless, given that the LISI was a more critical parameter in cases with vascular invasion than other liver scores, it was plausible that it is a stronger predictor of intrahepatic metastasis. Further studies are needed to determine whether the index in living liver donors is directly applicable to patients with HCC; however, the LISI stratifies the risk of HCC recurrence and is more sensitive than other liver function and nutritional indexes in patients at high risk of recurrence.

This study has several limitations that should be acknowledged. The analysis did not fully capture the function of other immune cells, including T cells, innate lymphoid cells, and natural killer T cells. It was imperative that the pathogenesis and prognosis through immunostaining are elucidated. However, the scarcity of TRAIL-positive NK cells in liver lymphocytes posed a significant challenge in evaluating this parameter. This study enrolled all Japanese patients, and it is also necessary to determine the effect of a higher BMI (>30%) on liver NK cells in other populations. Furthermore, a discussion on whether the index in living liver donors is directly applicable to patients with HCC is needed by evaluation of the function of liver NK cells in the resected liver. LISI in this study was constructed from donor livers exhibiting relatively mild to normal liver fibrosis. Consequently, the LISI, including the Fib-4 index, exhibited a pronounced elevation in HCC liver resection patients with severe liver fibrosis. To bridge this gap, it was essential to perform LISI validation in HCC liver resection patients and to reaffirm the validity of the revised score.

5 | CONCLUSIONS

Our model facilitated the prediction of RR among high-risk patients by providing LISI to predict the anti-tumor effects of NK cells.

AUTHOR CONTRIBUTIONS

YI and MO conceived and designed the study. YI, MO, IC, BT, KI, KS, MD, NR, RY, FH acquired the data and did all experiments. YI and MO analyzed and interpreted the data. YI and MO drafted the manuscript. YI, MO, NR, RY, SK, HT, KI, KI TK, YT, and HO critically revised the article. YI, MO, IC, BT, KI, KS, MD, NR, RY, FH, SK, HT, KI, KI, TK, YT, and HO approved the final version of the manuscript to be published.

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CONFLICT OF INTEREST STATEMENT

Hideki Ohdan is an editorial board member.

ETHICS STATEMENTS

Approval of the research protocol: The study protocol was approved by the ethics committee of our hospital (E-186, E-3459, E-1937).

Informed consent: The study conforms to the provisions of the Declaration of Helsinki. The need for written informed consent was waived owing to the retrospective nature of the study. The opt-out method to obtain patient consent was utilized.

Registry and the registration no. of the study/trial: N/A.

Animal studies: N/A.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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