High Serum Interleukin-34 Level is a Predictor of Poor Prognosis in Patients with Non-viral Hepatocellular Carcinoma

Yu Noda[1], Takumi Kawaguchi[1], Masaaki Korenaga[2], Sachiyo Yoshio[2], Sho Komukai[3], Masahito Nakano[1], Takashi Niizeki[1], Hironori Koga[1,4], Atsushi Kawaguchi[5], Tatsuya Kanto[2], Takuji Torimura[1,4]

1) Division of Gastroenterology, Kurume University School of Medicine, Kurume, Japan.
2) The Research Center for Hepatitis and Immunology, National Center for Global Health and Medicine, Ichikawa, Japan.
3) Division of Biomedical Statistics, Department of Integrated Medicine, Graduate School of Medicine, Osaka University, Osaka, Japan
4) Liver Cancer Research Division, Research Center for Innovative Cancer Therapy, Kurume University, Kurume, Japan.
5) Center for Comprehensive Community Medicine, Faculty of Medicine, Saga University, Saga, Japan.

Short title: Prognostic impact of IL-34 in non-viral HCC

Corresponding Author

Takumi Kawaguchi, M.D., Ph.D.

Division of Gastroenterology, Department of Medicine, Kurume University School of Medicine

67 Asahi-machi, Kurume 830-0011, Japan

Tel: +81-942-31-7627, Fax: +81-942-31-2623

E-mail: takumi@med.kurume-u.ac.jp

Abbreviations: HCC, hepatocellular carcinoma; IL-34, interleukin–34; HCC, hepatocellular carcinoma; TAMs, tumor-associated macrophages; HBV, hepatitis B virus; HCV, hepatitis C virus; AFP, α-fetoprotein; DCP, des-γ-carboxy prothrombin, BMI, body mass index, AST; aspartate aminotransferase, ALT, alanine aminotransferase; RFA, radiofrequency ablation; TACE, trans-arterial chemoembolization; HAIC, hepatic trans-arterial infusion chemotherapy; HR, hazard ratio; CI, confidence interval.

Abstract

Aims: We aimed to investigate the impact of interleukin (IL)-34 and YKL-40, regulators of hepatic fibrosis and tumor growth, on the prognosis of patients with non-viral hepatocellular carcinoma (HCC).

Methods: We enrolled 159 non-viral HCC patients (age, 70.8 ± 8.5 years; female/male, 43/116). Of these, 86 patients were alive and 73 patients had died at the censor time point. Serum IL-34 and YKL-40 levels were quantified by enzyme-linked immunosorbent assay. Patients were stratified by the median level of serum IL-34 to examine its effect on survival. Multivariate analysis and random forest analysis were employed to evaluate the impact of IL-34 and YKL-40 on the prognosis of non-viral HCC patients.

Results: IL-34 (HR 1.30, 95% CI 1.13-1.49, $P < 0.01$), tumor size (HR 1.63, 95% CI 1.37-1.94, $P < 0.01$), and tumor number (HR 1.53, 95% CI 1.25-1.87, $P < 0.01$) were independent predictive factors for survival. Furthermore, the survival rates were significantly lower in the high IL-34 group than in the low IL-34 group (5-year survival rates: 34.7% vs. 59.8%; $P < 0.05$). In the random forest analysis for survival, IL-34 was the third-highest ranking factor, following tumor size and number. In a stratification analysis, serum AFP level and FIB-4 index were independent positive risk factors for high serum IL-34 level. YKL-40 was not associated with prognosis in either the multivariate or random forest analysis.

Conclusion: IL-34 was an independent factor for survival of non-viral HCC patients. IL-34 may be associated with the prognosis through tumor and hepatic fibrosis factors.

Keywords: interleukin-34; YKL-40; hepatoma; survival; non-hepatitis B and non-hepatitis C virus; exploratory data analysis

Introduction

In recent years, the prevalence of non-hepatitis B virus-, non-hepatitis C virus-related hepatocellular carcinoma (non-viral HCC) has increased markedly [1-4]. The main etiologies of non-viral HCC are alcoholic liver disease, autoimmune hepatitis, and non-alcoholic steatohepatitis [5]. Hepatic fibrosis and its' biomarker such as wisteria floribunda agglutinin-positive Mac-2 binding protein are a risk factors for hepatocarcinogenesis [6, 7]. There are a lot of previous reports, which demonstrate biomarkers associated with prognosis of HCC [8-11]. However, limited information is available for biomarkers associated with prognosis of patients with non-viral HCC.

We previously reported that interleukin-34 (IL-34) and YKL-40 are associated with hepatic fibrosis in patients with non-alcoholic fatty liver disease [12, 13], which is one of the major etiologies of non-viral HCC. IL-34, a fibroblast-derived cytokine, promotes collagen synthesis by hepatic stellate cells [12], and elevated serum IL-34 level is found in patients with chronic inflammation, including obesity, and insulin resistance [14]. IL-34 is also involved in the differentiation and survival of macrophages in response to inflammation [15]. Furthermore, IL-34 is involved in the differentiation of tumor-associated macrophages (TAMs), which is associated with progression in various malignancies [16]. IL-34 overexpression is associated with tumor progression and lung metastasis in osteosarcoma patients [17]. IL-34 is reported to promote growth and metastasis of HCC through recruitment and infiltration of TAMs in both *in vitro* and *in vivo* studies [18]. Zhou et al. reported that high IL-34 level is associated with poor prognosis of patients with HCC [18]; however, no data is presented for

etiology of HCC. Thus, the clinical significance of IL-34 in the prognosis of non-viral HCC patients remains unclear.

YKL-40, also known as chitinase 3-like-1 or HC-gp39, was originally discovered as a secreted protein from a human osteosarcoma cell line [19], and is expressed in non-malignant cells [20]. YKL-40 is involved in the development of hepatic fibrosis [13], and elevated serum YKL-40 levels, which are associated with poor prognosis, have been described in patients with breast cancer, colorectal cancer, cholangiocellular carcinoma, and pancreatic cancer [21-24]. Although high serum YKL-40 levels are also associated with poor prognosis in HCC patients who undergo hepatic resection, the majority of enrolled patients in that study had hepatitis B virus (HBV)- or hepatitis C virus (HCV)-related HCC, which has different characteristics than non-viral HCC [25]. Thus, the clinical significance of YKL-40 in the prognosis of non-viral HCC patients remains unclear.

The aim of this study is to investigate the impact of IL-34 and YKL-40 on the prognosis of patients with non-viral HCC.

Methods

Study design and ethics

This is a retrospective study to investigate the impact of IL-34 and YKL-40 on the prognosis of patients with non-viral HCC. This protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki, as reflected by the prior approval of the institutional review board of Kurume University. An opt-out approach was used to obtain informed consent from the patients, and personal information was protected during data collection.

Subjects

We enrolled a total of 159 consecutive adult patients diagnosed with non-viral HCC at the Kurume University Hospital from January 1, 2005 to December 31, 2015. Non-viral HCC was defined as primary HCC that was negative for serum hepatitis B surface antigen and anti-hepatitis C antibody [26, 27]. HCC was diagnosed on the basis of histological examination or a combination of serum tumor markers, such as α-fetoprotein (AFP) and des-γ-carboxy prothrombin (DCP), and imaging modalities, such as dynamic computed tomography and dynamic magnetic resonance imaging, according to the Japanese Clinical Practice Guidelines for HCC [28]. The exclusion criteria were as follows: (1) younger than 20 years old, (2) history of treatment for HCC, (3) observational period less than 90 days, and (4) negative result of hepatitis B surface antigen due to anti-viral treatment for HBV.

The etiologies of the 159 enrolled patients with non-viral HCC were alcoholic liver disease (n = 67), autoimmune hepatitis (n = 10), non-alcoholic

steatohepatitis (n = 7), primary biliary cholangitis (n = 3), and cryptogenic liver disease (n = 72).

Enrolled patients were followed-up until March 31, 2016. The observational period was defined as the time span from the first treatment for non-viral HCC to death or the end of the study.

Data collection

The following data were collected at the time of diagnosis of non-viral HCC: host factors: age; sex; body mass index (BMI); alcohol intake (≥60 g/day, <60 g/day but >20 g/day, or ≤ 20 g/day); smoking (pack-years); history of diabetes, hypertension, or dyslipidemia; Child-Pugh score/class; white blood cell count; hemoglobin level; platelet count; prothrombin (PT) activity; serum levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), albumin, total bilirubin, triglyceride, sodium, C-reactive protein, hemoglobin A1c, hyaluronic acid, and creatinine; and FIB-4 index, which was calculated using the following formula: age (years) × AST [U/L]/(platelets [10^9/L] × (ALT [U/L]$^{1/2}$) [29]; tumor factors; size and number of tumors; macrovascular invasion; serum levels of AFP and DCP; and clinical staging (tumor-node-metastasis classification), based on the criteria of the Liver Cancer Study Group of Japan [30]; and treatment modality: hepatic resection, radiofrequency ablation (RFA), trans-arterial chemoembolization (TACE), or hepatic trans-arterial infusion chemotherapy (HAIC). Treatments were selected according to the clinical practice guidelines for HCC of the Japan Society of Hepatology [28].

Assessment of serum IL-34 and YKL-40 levels

Serum levels of IL-34 and YKL-40 were quantified using frozen serum collected at the time of diagnosis of non-viral HCC using an enzyme-linked immunosorbent assay kit (IL-34: R&D Systems, Minneapolis, MN, USA; YKL-40: Quidel, San Diego, CA, USA) as previously described [12, 13].

Statistical analysis

Data are expressed as a number, percentage, or mean ± standard deviation. Patients were classified into Alive and Deceased groups, and differences between the groups were analyzed using the Mann–Whitney *U*-test and chi-squared test, as appropriate. All P values were 2-tailed, and a P value < 0.05 was considered statistically significant. Variables associated with the survival of patients with non-viral HCC and elevation of serum IL-34 and YKL-40 levels were analyzed by data mining techniques using the software environment for statistical computing R (http://www.rproject.org/index.html). All statistical analyses were conducted by biostatisticians (SK and AK). The statistical methods are described in detail below.

Kaplan–Meier analysis

Patients were classified into a high IL-34 group or a low IL-34 group, based on the median value. The overall survival of all patients and each group was estimated using the Kaplan–Meier method, and differences in survival between the high IL-34 and low IL-34 groups were analyzed using the log-rank test.

Multivariate analysis

Multiple regression analysis using the forced entry method was used to investigate the importance of serum levels of IL-34 and YKL-40 and the FIB-4 index for survival in patients with non-viral HCC, as well as the factors related to high serum levels of IL-34 and YKL-40 in patients with non-viral HCC. Explanatory variables were selected in a stepwise manner. Data are expressed as hazard ratios (HRs) and 95% confidence intervals (CIs).

Random forest analysis

A random forest analysis was used to identify factors that distinguished the Alive and Deceased groups, as previously described [31]. The variable importance value, which reflects the relative contribution of each variable to the model, was estimated by randomly permuting its values and recalculating the predictive accuracy of the model.

Results

Patients' characteristics

The characteristics of the patients in the Alive (n = 86) and Deceased (n = 73) groups are summarized in Table 1. There were no significant differences in age, gender, BMI, or FIB-4 index. However, tumor size was significantly larger and tumor number was significantly higher in the Deceased group than in the Alive group. Moreover, hemoglobin level, platelet count, serum albumin level, and serum sodium level were significantly lower in the Deceased group than in the Alive group, and serum levels of ALT, C-reactive protein, and hyaluronic acid were significantly higher in the Deceased group than in the Alive group. There was no significant difference in serum YKL-40 level between the Alive and Deceased groups; however, serum IL-34 level was significantly higher in the Deceased group than in the Alive group (Table 1).

Kaplan–Meier analysis for survival in all patients

The median follow-up period of the entire cohort was 2.73 ± 2.34 years. Overall survival rates are presented in Figure 1; the median survival was 4.18 years. The 1-, 3-, 5-year survival rates were 84.8%, 59.4% and 44.5%, respectively (Figure 1).

Multivariate analysis for prognosis in patients with non-viral HCC

The results of the multivariate analysis for prognosis are summarized in Table 2. Independent positive risk factors for prognosis included tumor size, tumor number, hyaluronic acid level, and presence of hypertension. The

independent negative risk factors for prognosis were hemoglobin level (Table 2).

Although serum YKL-40 level and FIB-4 index were not significant factors associated with prognosis, serum IL-34 level was identified as a positive risk factor for the prognosis in patients with non-viral HCC (Table 2).

Kaplan–Meier analysis for survival according to serum IL-34 level

The Kaplan–Meier analysis for survival according to serum IL-34 level is shown in Figure 2. The survival rates of the high IL-34 group were significantly lower than those of the low IL-34 group. In the high IL-34 group, the 1-, 3-, and 5-year survival rates were 79.3%, 51%, and 34.7%, respectively. In the low IL-34 group, the 1-, 3-, and 5-year survival rates were 92.5%, 71.7%, and 59.8%, respectively (Figure 2).

We also examined the impact of IL-34 on the recurrence of HCC in patients treated with hepatic resection and RFA; however, there was no significant difference in the recurrence rate between the high IL-34 and low IL-34 groups (Supplementary figure 1).

Random forest analysis for survival

The results of the random forest analysis for survival are shown in Figure 3. The top distinguishable factors for survival were tumor size, tumor number, serum IL-34 level, hemoglobin level, hyaluronic acid level, presence of hypertension, treatment for HCC, and ethanol consumption (Figure 3).

Multivariate analysis for high serum IL-34 and YKL-40 levels

In multivariate analysis for serum IL-34 levels, PT activity was identified as a negative risk factor, and serum AFP level and FIB-4 index were identified as positive risk factors for high serum IL-34 level (Table 3).

In multivariate analysis for serum YKL-40 levels, serum levels of albumin and sodium were identified as negative risk factors, and FIB-4 index was identified as a positive risk factor for high serum YKL-40 level (Table 4).

Discussion

We demonstrated that high serum IL-34 level, but not serum YKL-40 level, was an independent risk factor for survival in patients with non-viral HCC. Prognosis was poorer in patients with high serum IL-34 level than in patients with low serum IL-34 level. In addition, random forest analysis revealed that the serum IL-34 level was the third factor for distinguishing between the Alive and Deceased groups. Furthermore, IL-34 levels were independently associated with liver fibrosis, as well as with tumor factors.

In the present study, YKL-40 was not an independent risk factor for prognosis of patients with non-viral HCC. However, previous studies have noted different results. Wan et al. performed a meta-analysis of survival rates in 10 clinical trials of breast cancer and reported that elevated YKL-40 expression is associated with poor overall and disease-free survival [22]. Chen et al. also reported that YKL-40 overexpression predicts poor prognosis and advanced tumor stage in patients undergoing curative resection of pancreatic cancer [21]. In addition, Zhu et al. reported that serum YKL-40 level is an independent prognostic factor for overall and recurrence-free survival in HCC patients receiving curative resection [25]. The reasons for the difference between our results and those of these previous reports are not clear. An elevation of serum YKL-40 level was associated with elevated serum levels of albumin and sodium and FIB-4 index; however, elevation of serum YKL-40 levels was not associated with tumor factors, such as tumor size and number, or serum levels of AFP and DCP in this study. In our previous study, tumor factors were the most significant factors associated with prognosis of patients with non-viral HCC [32]. Since YKL-

40 level was not associated with tumor factors, serum YKL-40 level might not be associated with the prognosis of patients with non-viral HCC in this study.

In the present study, serum IL-34 level was an independent risk factor for prognosis of patients with non-viral HCC. Baghdadi et al. reported that high expression of IL-34 was associated with poor survival in patients with lung cancer [33], and Zhou et al. reported that high IL-34 levels indicated a poor prognosis with shorter overall survival in patients with HCC after curative resection [18]. Therefore, our results are in good agreement with those of previous reports. In addition, we first demonstrated that, even in patients with non-viral HCC after non-curative therapy, elevated serum IL-34 levels were associated with poor prognosis. Moreover, we investigated the prognostic factors of patients with non-viral HCC by random forest analysis and found that elevated serum IL-34 level was the third factor from the top, following tumor size and number. Thus, serum IL-34 level was an important prognostic factor in patients with non-viral HCC, regardless of which type of treatment patients received.

We examined factors contributing to high serum IL-34 level and found that PT activity, FIB-4 index, and serum AFP levels were independently associated with serum IL-34 levels. PT activity and FIB-4 index are markers for liver function and hepatic fibrosis, respectively. We previously reported an association of hepatic fibrosis and serum IL-34 level in patients with NAFLD [12]. In this study, the prevalence of Child-Pugh class B and C was high in the high IL-34 group compared to the low IL-34 group (data not shown). Preisser et al. reported that, in patients with HCV or HBV infection, injured hepatocytes

secrete IL-34, which induces monocyte recruitment and differentiation into profibrogenic macrophages, as well as promoting type I collagen secretion by hepatic stellate cells [34, 35]. Thus, IL-34 may be associated with poor prognosis via hepatic decompensation. In addition, IL-34 is reported to be involved in the differentiation of TAMs, which is associated with progression in various malignancies [16]. IL-34 promotes the growth and metastasis of HCC through recruitment and infiltration of TAMs [16]. Moreover, IL-34 expression is known to be associated with therapeutic resistance in lung cancer and melanoma [33, 36, 37]. Therefore, in this study, serum IL-34 level may reflect hepatic fibrosis as well as tumor factors, and was associated with the prognosis of patients with non-viral HCC.

There are limitations in this study. First, we did not enroll patients with viral-related HCC and, therefore, it remains unclear that an association between prognosis of HCC and IL-34 or YKL-40 is specific for patients with non-viral HCC. Second, the predictive ability of IL-34 on prognosis of non-viral HCC was not compared to other prognostic biomarkers for patients with HCC including miR-128-3p, PD-1+ T cells [8, 9]. Advantage and disadvantage of IL-34 should be examined in the further study.

In conclusion, we demonstrated that high serum IL-34 levels were associated with poor prognosis in patients with non-viral HCC. In addition, data mining analysis revealed that serum IL-34 level was the third-highest prognostic factor for patients with non-viral HCC. Moreover, IL-34 was independently associated with liver fibrosis and tumor factors. Hence, regulation of IL-34 may lead to better prognosis for patients with non-viral HCC.

Disclosure Statement

Takumi Kawaguchi received lecture fees from Mitsubishi Tanabe Pharma Corporation. The other authors have no conflicts of interest.

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Author Contributions

YN and TK participated in study conception and design, acquisition of data, interpretation of data, and drafting of manuscript. MK, SY, MN, and TN participated in acquisition of data and interpretation of data. SK and AK participated in analysis and interpretation of data. HK, TK and TT participated in study conception, design, and critical revision.

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Table 1. Patients' characteristics

Variables	Alive group (N = 86)	Deceased group (N = 73)	P
Age, y	70.4 ± 8.91	71.2 ± 8.03	0.60
Gender (Female/Male)	23 (26.7)/63 (73.3)	20 (27.4)/53 (72.6)	0.93
Body mass index, kg/m^2	24.3 ± 3.4	24.7 ± 3.6	0.45
Ethanol consumption			0.51
< 20 g/day	36 (42.9)	29 (43.3)	
20–60 g/day	11 (13.1)	13 (19.4)	
≥ 60 g/day	37 (44.0)	25 (37.3)	
Smoking (pack-years)	31.1 ± 35.9	19.9 ± 25.0	0.18
Diabetes mellitus	39 (45.4)	34 (46.6)	0.88
Etiology			0.32
Autoimmune hepatitis	6 (6.99)	4 (5.48)	
Non-alcoholic steatohepatitis	5 (5.81)	2 (2.74)	
Primary biliary cholangitis	3 (3.49)	0	
Alcoholic liver disease	39 (45.35)	28 (38.36)	
Cryptogenic liver disease	33 (38.37)	39 (53.4)	
Child-Pugh class			0.47
A	69 (80.2)	53 (72.6)	
B	16 (18.6)	18 (24.7)	
C	1 (1.2)	2 (2.7)	
Tumor size (mm)	40.3 ± 37.9	53.3 ± 34.0	0.03
Tumor number	2 ± 2	4 ± 3	< 0.001
Macrovascular invasion	11 (12.8)	14 (19.18)	0.27

Variables	Alive group (N = 86)	Deceased group (N = 73)	P
HCC stage			0.01
I	10 (11.6)	3 (4.1)	
II	48 (55.8)	30 (41.1)	
III	21 (24.4)	21 (28.8)	
IVA	5 (5.8)	13 (17.8)	
IVB	2 (2.4)	6 (8.2)	
Treatment for HCC			0.08
Hepatic resection	24 (27.9)	11 (15.1)	
RFA	28 (32.5)	20 (27.4)	
TACE	22 (26.6)	23 (31.5)	
HAIC	11 (14.0)	19 (26.0)	
Hypertension	58 (67.4)	41 (56.2)	0.14
Dyslipidemia	22 (25.6)	7 (9.6)	0.02
White blood cell count (/mm^3)	4,766 ± 1,472	5,088 ± 3,544	0.59
Hemoglobin (g/dL)	13.0 ± 1.8	12.3 ± 1.7	0.03
Platelet count (10^4/mm^3)	13.9 ± 5.5	12.1 ± 7.5	0.03
AST (IU/L)	42.2 ± 19.7	47.9 ± 27.4	0.32
ALT (IU/L)	34.4 ± 23.6	41.6 ± 24.5	0.01
Albumin (g/dL)	3.74 ± 0.53	3.51 ± 0.43	0.002
Total bilirubin (mg/dL)	1.00 ± 0.48	1.18 ± 0.70	0.12
Prothrombin activity (%)	82.8 ± 17.8	82.1 ± 15.6	0.63
Sodium (mmol/L)	140.2 ± 3.0	138.9 ± 3.1	0.002
Creatinine (mg/dL)	0.77 ± 0.26	0.87 ± 0.75	0.42
Triglyceride (mg/dL)	105.8 ± 45.4	95 ± 43	0.295

Variables	Alive group (N = 86)	Deceased group (N = 73)	P
CRP (mg/dL)	0.42 ± 0.90	0.89 ± 1.62	0.04
AFP (ng/mL)	4,429 ± 25,667	6,599 ± 25,856	0.08
DCP (mAU/mL)	5,827 ± 16,028	7,028 ± 17,206	0.07
HbA1c (%)	6.19 ± 1.08	6.23 ± 1.16	0.99
Hyaluronic acid (ng/mL)	276.1 ± 353.1	441.2 ± 516.6	0.004
IL-34 (pg/mL)	10.8 ± 13.5	16.7 ± 28.1	0.005
YKL-40 (pg/mL)	703.3 ± 734.2	700.2 ± 499.5	0.14
FIB-4 index	4.58 ± 2.77	5.41 ± 3.17	0.07

Data are expressed as mean ± SD or n (%).

Abbreviations: HCC, hepatocellular carcinoma; RFA, radiofrequency ablation; TACE, transarterial chemoembolization; HAIC, hepatic arterial infusion chemotherapy; AST, aspartate aminotransferase; ALT, alanine aminotransferase; CRP, C-reactive protein; AFP, alpha-fetoprotein; DCP, des-γ-carboxy prothrombin; HbA1c, Hemoglobin A_{1c}; IL-34, Interleukin-34.

Table 2. Multivariate analysis for survival in patients with HCC

Variable	Group/Unit	HR	95% CI		P
			lower	upper	
YKL-40 (pg/mL)	10	1.00	1.00	1.01	0.2852
IL-34 (pg/mL)	10	1.30	1.13	1.49	0.0002
FIB-4 index	1	1.16	0.96	1.39	0.1185
Tumor size (mm)	10	1.63	1.37	1.94	< 0.0001
Number of tumors	1	1.53	1.25	1.87	< 0.0001
Hemoglobin (g/dL)	1	0.52	0.37	0.73	0.0001
Hyaluronic acid (ng/mL)	10	1.01	1.00	1.02	0.004
Hypertension	None	0.14	0.04	0.52	0.0035
Ethanol consumption	> 60 g/day	0.39	0.14	1.10	0.074
Triglyceride (mg/dL)	1	1.01	1.00	1.02	0.0567

Note. Abbreviations: HCC, hepatocellular carcinoma; HR, hazard ratio; CI, confidence interval; IL-34, interleukin-34.

Table 3. Multivariate analysis for high serum IL-34 levels

Variable	Unit	HR	95% CI		P
			lower	upper	
PT activity (%)	10	0.48	0.35	0.65	< 0.0001
AFP (ng/mL)	1,000	1.04	1.01	1.06	0.0011
FIB-4 index	1	1.39	1.18	1.64	< 0.0001

Note. Abbreviations: IL, interleukin; HR, hazard ratio; CI, confidence interval; PT, prothrombin; AFP, alpha fetoprotein.

Table 4. Multivariate analysis for high serum YKL-40 levels

Variable	Unit	HR	95% CI		P
			lower	upper	
Albumin (g/dL)	0.1	0.92	0.86	0.99	0.0363
Sodium (mmol/L)	1	0.85	0.75	0.96	0.0071
FIB-4 index	1	1.22	1.07	1.40	0.0025

Abbreviations: HR, hazard ratio; CI, confidence interval

Figure legend

Figure 1. Kaplan–Meier analysis for survival in all patients. The median survival was 4.18 years in non-viral HCC patients. The 1-, 3-, 5-year survival rates were 84.8%, 59.4%, and 44.5%, respectively.

Figure 2. Kaplan–Meier analysis for survival according to serum interleukin (IL)-34 level. The median survivals were 6.07 years in the low IL-34 group and 3.45 years in the high IL-34 group. The high IL-34 group showed significantly poorer prognosis than the low IL-34 group (1-, 3-, and 5-year survival rates; 79.3% vs. 92.5%, 51% vs. 71.7%, and 34.7% vs. 59.8%, respectively; P = 0.0094 for all).

Figure 3. Random forest analysis for survival. The top distinguishable factors for survival were tumor size, tumor number, serum IL-34 level, hemoglobin, hyaluronic acid, presence of hypertension, type of treatment for hepatocellular carcinoma, and ethanol consumption.

Supplementary figure 1. Difference in recurrence rate between the high IL-34 and low IL-34 groups in the HCC patients treated with hepatic resection and RFA.

Figure 1

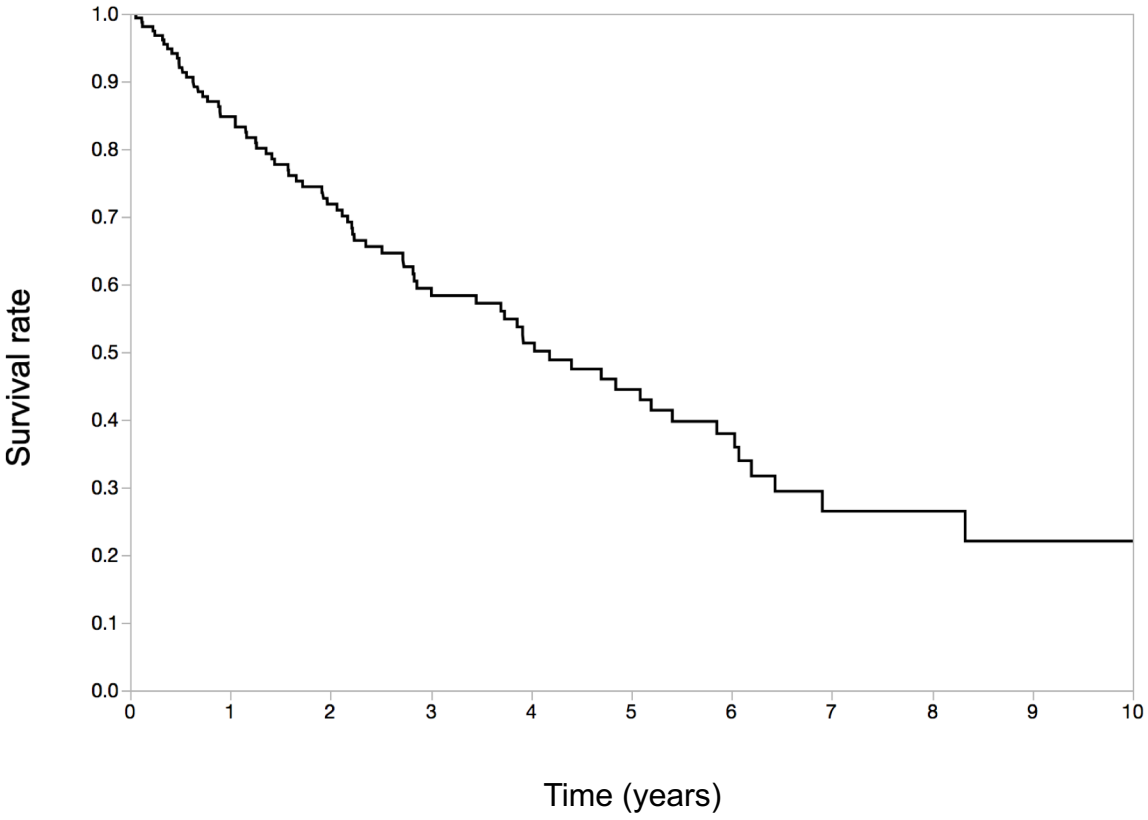

Survival rate
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
0
1
2
3
4
5
6
7
8
9
10
Time (years)

Figure 2

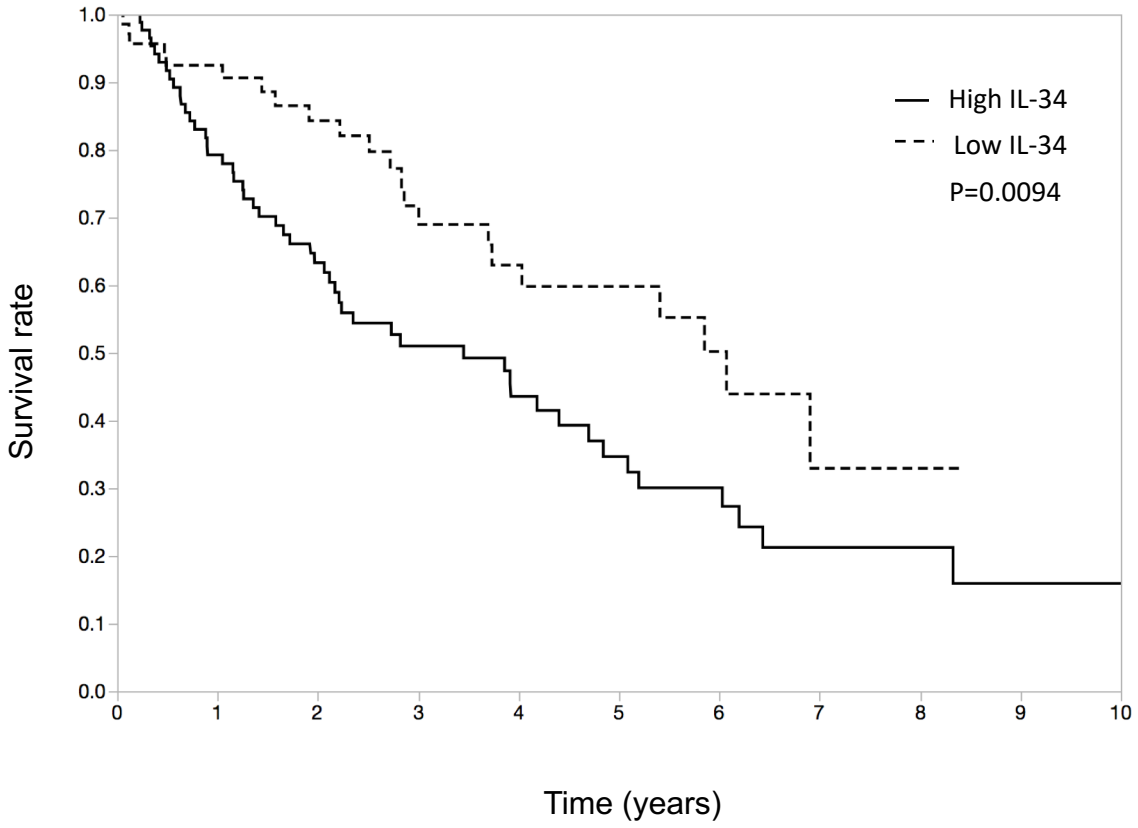
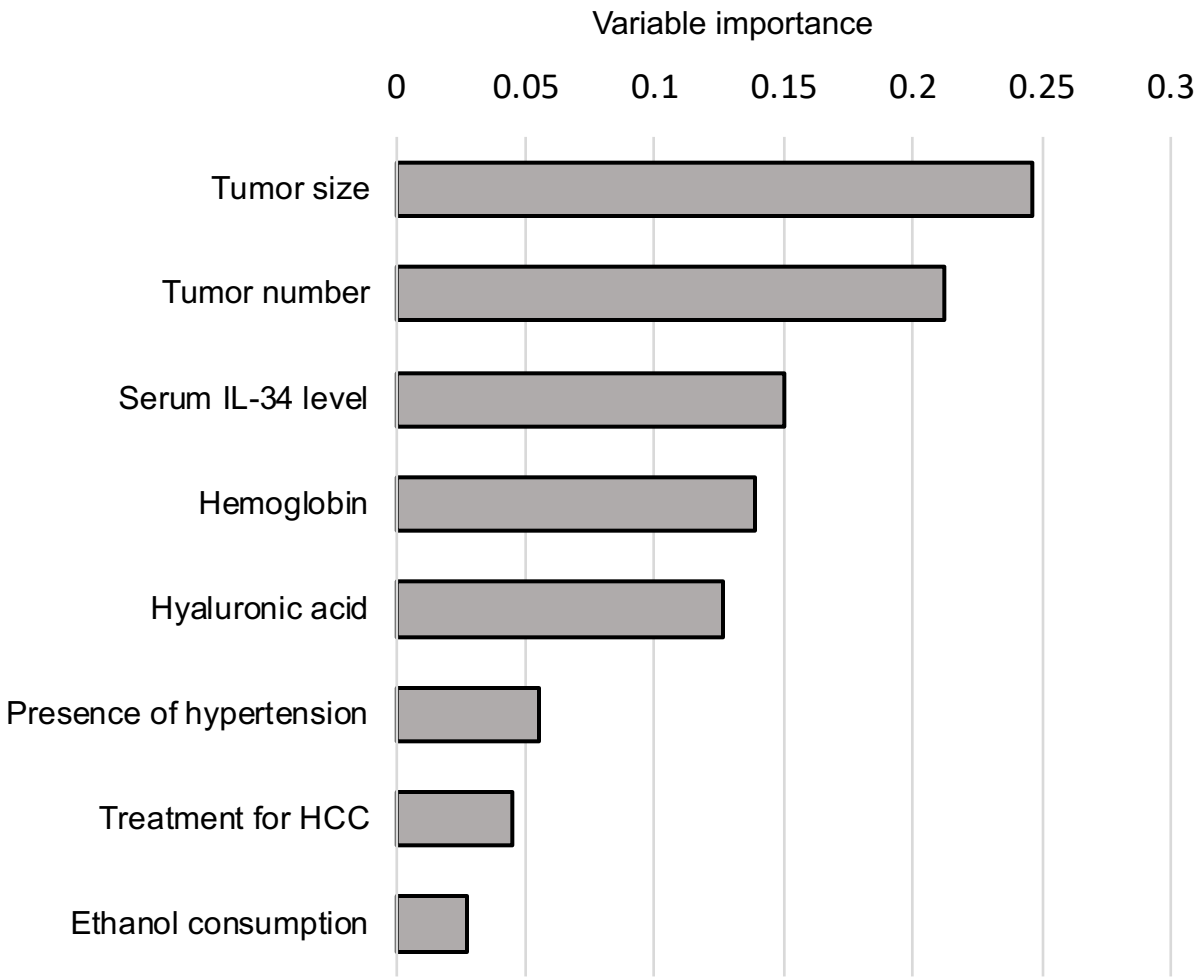

1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
Survival rate
0
1
2
3
4
5
6
7
8
9
10
Time (years)
High IL-34
Low IL-34
P=0.0094

Figure 3


Variable importance
0
0.05
0.1
0.15
0.2
0.25
0.3
Tumor size
Tumor number
Serum IL-34 level
Hemoglobin
Hyaluronic acid
Presence of hypertension
Treatment for HCC
Ethanol consumption

Supplementary figure 1