Superiority of CRP-Albumin-Lymphocyte index (CALLY index) as a non-invasive prognostic biomarker after hepatectomy for hepatocellular carcinoma

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Abstract

Background: We aimed to investigate whether a novel biomarker incorporating albumin, lymphocytes, and CRP can predict the prognosis for hepatocellular carcinoma (HCC) after hepatectomy.

Methods: Between January 2011 and December 2013, 384 patients who underwent hepatectomy in four university hospitals in Japan were investigated as a discovery cohort. The CRP-Albumin-Lymphocyte (CALLY index) was defined as (Albumin×Lymphocyte)/(CRP×10^4). Patients with a CALLY index ≥5 (n=200) were compared to those with an index <5 (n=184). Next, validation was performed using 267 patients from three other university hospitals (external validation cohort).

Results: The number of TNM Stage III and IV patients was significantly higher in the CALLY <5 group than the ≥5 group (p=0.003). There was a significant difference in the 5-year survival rate (CALLY ≥5: 71% vs. <5: 46%; p<0.001). Multivariate analysis identified the CALLY index as an independent factor of overall survival. Similarly, there was a significant difference in the 5-year survival rate between the CALLY ≥5 (73%) and <5 (48%) groups (p<0.001), and the CALLY index was identified as an independent prognostic factor in the external validation cohort.

Discussion: The CALLY index derived from CRP, albumin, and lymphocyte values is a promising predictive biomarker for postoperative prognosis of patients with HCC.

Introduction

Non-invasive prognostic biomarkers identified using a range of standard test results have recently attracted attention among researchers. The Glasgow prognostic score (GPS), proposed in a study investigating non-small-cell lung carcinoma (NSCLC) that was published in 2003, is one such novel biomarker. It has been suggested that the GPS is superior to other biomarkers based on the staging or performance status of the disease.[1] Recently, numerous studies have described the neutrophil-lymphocyte ratio (NLR), which was initially reported as a predictive index for the prognosis of critically ill hospitalized patients, as a predictive marker for cancer prognosis.[2-6] The platelet-lymphocyte ratio (PLR) has also increasingly drawn attention as a biomarker for cancer prognosis because thrombocytosis is often observed in cases of solid carcinoma with chronic inflammation, and thrombocytes are closely associated with cancer progression.[7, 8] Several studies have reported a correlation between these markers and the prognosis of patients who underwent a hepatectomy for hepatocellular carcinoma (HCC).[9-16] Using various blood test data, many new biomarkers have been established and their significance for enabling a readily obtainable prognostic prediction is indisputable. However, there is a need for the establishment of further novel biomarkers that have better precision. In this study, we sought to establish a novel non-invasive biomarker for the prognosis of HCC using data available in multiple medical facilities.

To this end, we focused on two representative markers: the GPS and the prognostic nutrition index (PNI). The GPS is a system that evaluates nutritional status and inflammatory reactions using a combination of the serum C-reactive

protein (CRP) and the serum albumin values. It classifies subjects into three groups, with serum CRP and albumin cut-off values of 1.0 mg/dL and 3.5 mg/dL, respectively.[1] The modified GPS (mGPS), with a CRP cut-off value of 0.5 mg/dL, is often applied to Japanese patients.[17]

PNI is a nutrition index related to prognosis which was initially calculated using a formula taking into consideration the serum albumin value, sebum thickness of the triceps brachii muscle, serum transferrin value, and delayed skin hypersensitivity reaction.[18] As this formula was complicated and made calculations difficult, Onodera et al. proposed a simplified PNI formula using only serum albumin value and total lymphocyte count.[19] Both indices were developed to predict perioperative complication risks by evaluating the preoperative PNI, and to assist in determining the optimal timing for surgical treatment.

In cancer patients, albumin values and lymphocyte counts are reduced, and CRP values are elevated due to poor nutrition, weakened immune system, and exacerbation of inflammation. Therefore, in this study, we aimed to develop and validate a CRP-albumin-lymphocyte index (CALLY index) as a prognostic biomarker for HCC after hepatectomy.

Methods

This multicenter, retrospective study conformed to the Clinical Research Guidelines and was approved by the ethical committee of each institution (approval number in principal institution: 29-086). Informed consent was obtained by opt-out from all patients or members of their families at each institution.

Discovery cohort

In the discovery cohort, we enrolled 384 patients who underwent hepatectomy for HCC at Shiga University of Medical Science Hospital, Kansai Medical University Hospital, Osaka Medical College Hospital, or Nara Medical University Hospital between January 1, 2011 and December 31, 2013. Patients who underwent a combined resection of other organs or had residual tumor were excluded from this study.

The patient background factors investigated were age, sex, body mass index (BMI), presence/absence of diabetes, history of alcohol abuse, and hepatitis virus infection. The blood test parameters examined were white blood cell (WBC) count, lymphocyte count, platelet count, albumin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), total bilirubin, prothrombin activity (PT), CRP, indocyanine green retention rate at 15 minutes (ICGR15), and Child-Pugh classification. The tumor and surgical factors examined included maximum tumor size, tumor number, TNM 8^{th} ed. classification, α-fetoprotein (AFP), protein induced by vitamin K absence or antagonist-II (PIVKA-II), surgical methods, surgical duration, and blood loss.

We defined the CALLY index as the preoperative albumin value multiplied by the lymphocyte count and divided by the CRP value multiplied by 10,000 [(Albumin×Lymphocyte) /(CRP×10^4)]. The CALLY index cut-off value was set to 5, which was the most clinically applicable value according to receiver operating characteristic (ROC) analysis. Patients with a CALLY index ≥5 (n = 200) were compared with those with a CALLY index <5 group (n = 184).

We then defined the CALLY score as albumin <3.5 g/dL, lymphocyte count <1000/μL, and CRP value >0.5 mg/dL, and stratified patients according to how many of these values they demonstrated in order to compare the rates of overall survival (OS) rate and recurrence-free survival (RFS).The patients were divided into the CALLY-S0 (n = 242), CALLY-S1 (n = 97), CALLY-S2 (n = 37), and CALLY-S3 (n = 8) groups.

Validation cohort

For the external validation cohort, we included 267 patients who underwent hepatectomy for HCC at Osaka City University Hospital, Kindai University Hospital, or Wakayama Medical University Hospital during the same period and performed the same analysis as described for the discovery cohort.

Statistical analysis

Values are presented as the mean ± standard deviation for age, BMI, and maximum tumor size, and as the median value and quartile range of all other variables. The survival rate was calculated by the Kaplan–Meier method using the log-rank test. The Cox proportional hazards model was used to perform multivariate analysis on factors with $p<0.05$ in the univariate analysis. The cut-off values of continuous variables were selected by ROC curve analysis. In addition, we chose the cut-off values that were close to the most significant difference and were easy to memorize and apply clinically. In all statistical analysis, p-values <0.05 were considered statistically significant. All statistical analyses were

performed with R version 3.6.2 (The R Foundation for Statistical Computing, Vienna, Austria; https://cran.r-project.org/bin/macosx/).

Results

Discovery cohort

Table 1 shows the comparison between background factors in the CALLY ≥5 group and CALLY <5 groups of the discovery cohort. The mean age was 69 years in both groups, while a significantly higher BMI was observed in the CALLY <5 group compared to the CALLY ≥5 group (23.1% vs. 23.8%; p = 0.045). The number of hepatitis B patients was the same between the two groups, while the number of hepatitis C patients was significantly higher in the CALLY ≥5 group compared to the CALLY <5 group (47% vs. 30.4%; p = 0.001).

The CALLY ≥5 group showed significantly higher albumin levels and lymphocyte counts but significantly lower CRP values compared to the CALLY <5 group. The platelet count was significantly lower in the CALLY ≥5 group (155,000/μL) than in the CALLY <5 group (179,000/μL) (p = 0.014). The CALLY <5 group showed significantly higher Child–Pugh class B classification compared to the CALLY ≥5 group (11.4% vs. 3.5%; p = 0.003).

The mean maximum tumor size was significantly larger in the CALLY <5 group (5.4 cm) than in the CALLY ≥5 group (3.5 cm) (p < 0.001). We also evaluated tumor progression according to the TNM 8th ed. staging system. The percentage of patients classified as stage III/ IV was 3.5% in the CALLY ≥5 group and 14.7% in the CALLY <5 group, and the difference was statistically significant (p = 0.003). The tumor markers AFP and PIVKA-II were both significantly higher in the CALLY

<5 group compared to the CALLY ≥5 group. There was no difference between the groups in operation time, blood loss, and surgical method (Table 1).

The 5-year survival rate was 71% in the CALLY ≥5 group and 46% in the CALLY <5 group, and the difference was statistically significant ($p < 0.001$). Similarly, for the 5-year RFS rate, there was a significant difference ($p < 0.001$) between the CALLY ≥5 group (38%) and the CALLY <5 group (24%) (Figure 1).

Tables 2 and 3 show the univariate and multivariate analysis of OS and RFS. Univariate analysis confirmed that the significant factors were presence/absence of hepatitis B surface (HBs) antigen, Child–Pugh classification, TNM classification, AFP values, PIVKA-II values, blood loss, and CALLY index. From the results of multivariate analysis using these factors, we identified presence/absence of HBs antigen, TNM classification, AFP values, PIVKA-II values, and CALLY index as independent prognostic factors. Similar to the above results, we identified TNM classification, AFP values, PIVKA-II values, and the CALLY index as factors contributing to RFS (Tables 2 and 3).

Figure 2 shows the comparison between different biomarkers reported to contribute to the prognosis of various cancers. ROC analysis revealed that the area under the curve (AUC) for the CALLY index was 0.666. We also assessed AUCs for the CRP-albumin ratio (CAR) (0.661), lymphocyte-CRP ratio (LCR) (0.663), NLR (0.587), PLR (0.57), and PNI (0.606); the AUC for the CALLY index was the most accurate (Figure 2).

Figure 3 shows the results of the CALLY score analysis. The 5-year survival rate of the CALLY-S0 group, which had no factors corresponding to albumin <3.5 g/dL, lymphocyte count <1000/μL, or CRP value >0.5 mg/dL, was 69%. The 5-year

survival rate of the CALLY-S1, -S2, and -S3 groups were 49%, 31%, and 0%, respectively ($p < 0.001$). The 5-year RFS rate of the CALLY-S0 group was 38%, while that of the CALLY-S1, -S2, and -S3 groups were 27%, 5%, and 0%, respectively ($p < 0.001$) (Figure 3).

External validation cohort

We conducted the same investigation over the same period at three other university hospitals using the patients assigned to the external validation cohort. Table 4 shows the comparison of background factors between the discovery cohort and the external validation cohort. Age, sex, and BMI were similar between the two cohorts, but the number of patients with diabetes was higher in the external validation cohort compared to the discovery cohort (37.1% vs. 28.9%; $p = 0.033$). The percentage of hepatitis C patients was 39.1% in the discovery cohort and 50.2% in the external validation cohort, and the difference was statistically significant ($p = 0.005$). A comparison of factors associated with liver function revealed that the platelet count was significantly lower, and the albumin values were higher, in the external validation cohort. Although the number of stage III/IV patients was significantly higher in the external validation cohort compared to the discovery cohort (18.4% vs. 8.9%; $p < 0.001$), the level of tumor markers was the same between the two groups (Table 4). The 5-year survival rate in the external validation cohort was 73% in the CALLY ≥5 group and 48% in the CALLY <5 group ($p < 0.001$). Similarly, the 5-year RFS rate was 43% in the CALLY ≥5 group and 26% in the CALLY <5 group ($p < 0.001$) (Figure 4).

Univariate analysis of prognosis confirmed that the significant factors were Child-Pugh classification, TNM classification, AFP value, PIVKA-II value, percentage of laparoscopic resection, operation time, blood loss, and CALLY index. From the results of multivariate analysis of these factors, we identified TNM classification and CALLY index as independent prognostic factors. We similarly identified WBC, TNM classification, blood loss, and CALLY index as prognostic factors for RFS (Tables 5 and 6).

In the analysis of the CALLY score in the external validation cohort, the stratification was similar to that of discovery cohort, except for the CALLY-S3 group, which had only two cases (Figure 5). The 5-year survival rate was 72% in the CALLY-S0, 48% in CALLY-S1, and 36% in CALLY-S2 groups ($p < 0.001$), and the five-year RFS rates were 41% in the CALLY-S0, 29% in CALLY-S1, and 18% in CALLY-S2 groups ($p < 0.001$).

Figure 6 shows the 5-year survival rate after combining the TNM classification and CALLY score in all cohorts (n = 651). We used all cohorts because there were few patients with TNM stage IV and patients with CALLY-S2 and -S3. The 5-year survival rate of CALLY-S0 was 74.7%, -S1 was 63.0%, and -S2≥ (S2 and 3) was 37.8% for stage IA+B of the TNM classification. The 5-year survival rate of CALLY-S0 was 68.0%, -S1 was 46.3%, and -S2≥ was 29.9% for stage II TNM classification. The 5-year survival rate for CALLY-S0 was 57.7%, -S1 was 24.4%, and -S2≥ was 11.4% for stage III TNM classification. Although the number of patients in the stage IV group was small, the 5-year survival rate for CALLY-S0 was 50.0%, and none of the patients in the CALLY-S1 and -S2≥ groups survived for 5 years.

Discussion

Malnutrition in cancer patients is generally related to cancer-associated weight reduction or to cancer-induced weight reduction.[20] Cancer-associated weight reduction is caused by nutrient absorption disorder due to constriction or occlusion in the digestive tract, or by anorexia induced by anticancer drugs or psychological stress. In contrast, cancer-induced weight reduction is caused by inflammatory cytokines that are actively secreted by cancer cells. These conditions progress together with the progression of cancer, worsening the levels of malnutrition or inflammation and eventually leading to death of the patient. Consequently, various indices that indicate the level of nutrition or inflammation level have drawn attention as potential biomarkers for the prediction of cancer prognosis, including the GPS and the PNI. Recently, several studies have reported their validity not only in the risk evaluation of perioperative complications but also as prognostic factors for various cancers.[21-26]

In this study, we designed a biomarker combining the mGPS and Onodera's PNI. We used the serum albumin value as the indicator of the patients' nutritional status and liver function, the lymphocyte count as the indicator of immune status, and the CPR value as the indicator of a patients' inflammation level. Albumin is a primary protein synthesized in the liver. Owing to its relatively long half-life, it is widely used not only for determining nutritional status but also for evaluating liver function. Albumin values are closely related to prognosis in HCC cases; thus, branched chain amino acid administration is recommended to improve prognosis.[27, 28] The lymphocyte count is widely used as an index of

immunocompetence, as lymphocytes play a role in tumor immunity to suppress carcinogenesis.[29] CRP is protein produced by the hepatocytes in response to stimulation by IL-6. When inflammation occurs in the body, due to cancer, infection, or physical trauma, a reaction called the acute phase response occurs to maintain homeostasis, and proteins that fluctuate in response to this reaction are called acute-phase proteins (APPs). CRP and albumin are representative APPs that fluctuate according to the exacerbation of cancer tissue inflammation; the concentration of the former is increased while that of the latter is decreased. The CALLY index designed in this study showed favorable results in reflecting prognosis. We also identified it as an independent prognostic factor in multivariate analysis, along with other common prognostic factors such as TNM classification and tumor markers.

Several other predictive markers of prognosis have been previously reported. NLR is one such biomarker, which incorporates the neutrophil and lymphocyte counts. Neutrophils are considered to be involved in cancer prognosis because, like CRP, their production increases with an increase in the level of inflammation, and they induce chemokine/cytokine production that promotes tumor cell proliferation, tumor invasiveness, and angiogenesis.[30-33] PLR is a biomarker similar to NLR, with neutrophil count replaced by platelet count. Platelet count often increases in patients with solid cancer showing chronic inflammation. Several studies have reported the validity of PLR as a biomarker, as platelets are closely associated with cancer progression.[8, 34, 35] There are also several studies reporting the validity of CAR, which uses albumin and CRP values, and LCR, which uses lymphocyte count and CRP value, as biomarkers.[36, 37] Comparison of

CALLY index with these prognostic predictive markers showed that CALLY index was the most effective in predicting prognosis of HCC patients. Further, we used only three factors in this study, and the calculation method used for the CALLY index was not complicated. Taken together, the accuracy and simplicity of this model suggests the superiority of this method in predicting the prognosis.

In the discovery cohort, using ROC analysis, the CALLY index cut-off value was set to 5, which was the most clinically applicable value. As the number of HCC patients with hepatitis C infection in Japan varies according to regions, the possibility of differences in the background factors between discovery and external validation cohorts cannot be denied. However, the validity of the CALLY index was confirmed even in the external validation cohort by setting the cut-off value as 5.

In addition, we categorized the subjects into four groups: CALLY-S0, -S1, -S2, and -S3, according to their alignment with the CALLY score corresponding to albumin <3.5 g/dL, lymphocyte count <1000/μL, and CRP value >0.5 mg/dL. We used the same cut-off values as the GPS for albumin (3.5 g/dL),[1] which was also used in the Child-Pugh score. We set the cut-off value of the lymphocyte count as 1,000/μL. In general, patients with lymphocyte count less than 1,000/μL are considered to be immunocompromised.[29] The CRP cut-off value was set as 0.5 mg/dL, which is the cut-off value of mGPS established by Onodera et al. for use in Japanese patients.[19] As a result, we could successfully stratify both the discovery and the external validation cohorts. The mGPS is also widely used in Asia and Europe, suggesting that the validity of the CALLY score might not be limited to Japan.[38, 39]

Here, we examined both the CALLY index and CALLY score, both of which could potentially be used as postoperative prognostic biomarkers for HCC. Among them, the CALLY index was considered to be more useful and accurate, since the number of cases with CALLY scores of S2 and S3 was small. Therefore, the CALLY score should be verified using a larger number of cases in the future.

Next, we will discuss the clinical use of this CALLY index and CALLY score. Indeed, surgical indications should be decided based on the degree of cancer progression, depending on tumor size, tumor number, and vascular invasion. However, patients with a low CALLY index and CALLY score may benefit from non-surgical treatments first, such as interventional radiology and chemotherapy. During the non-surgical treatment period, it would be preferable to improve their CALLY index and CALLY score by improving their nutritional condition and reducing inflammation. If their CALLY index and CALLY score improves, surgery should be performed, and if it does not improve, non-surgical treatments should be continued. In particular, we believe that the combination of molecular-targeted drugs and immune checkpoint inhibitors is a promising treatment strategy in patients with a low CALLY index and CALLY score. In addition, the group with a low CALLY index had significantly more postoperative complications (data not shown). Therefore, the CALLY index may also help determine surgical indications.

The TMN classification indicates the degree of tumor progression, and the CALLY index and score indicates the patient's nutritional condition. Since they consider completely different factors, they could be combined to improve the prognostic prediction. For colorectal cancer, the combination of the TNM classification and an immunological index, called the immune score, has been

shown to have higher prognostic accuracy than the TNM classification alone. Similarly, we found that the combination of the TNM classification and CALLY score predicted prognosis relatively well, as shown in Figure 6. However, since the number of patients with CALLY-S2 and -S3, as well as patients with TNM stage IV was small, the prognostic prediction ability of the TNM classification combined with the CALLY score should be further investigated with large number of patients in the future.

This study had certain limitations. The investigation only included Japanese patients and the number of patients was small. It should also be noted that we used three factors, which is different from other representative non-invasive biomarkers. Despite the use of three factors, the formula used is simple and allows easy calculation. Moreover, the accuracy of our biomarker was higher than that of other representative non-invasive biomarkers, suggesting its potential as an effective tool for prognostic predictions. However, further investigation with a sufficient number of patients, unresectable patients, or chemotherapy patients should be conducted.

In conclusion, the CALLY index and CALLY score, which are calculated using the CRP value, albumin level, and lymphocyte count, are promising predictive biomarkers for the postoperative prognosis of HCC patients.

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Not applicable.

Statement of Ethics

This multicenter, retrospective study conformed to the Clinical Research Guidelines and was approved by the ethical committee of each institution (approval number in principal institution: 29-086). Informed consent was obtained by opt-out from all patients or members of their families at each institution.

Disclosure Statement

None declared.

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Author Contributions

HI designed the research and analyzed the patient data. HI, MT, KK, TM, HM, ST, MU, TN, HM, HM, KM, FH, MK, and SK performed data collection. HI drafted the manuscript. All authors read and approved the final version of the manuscript.

Availability of data and materials

The datasets during and/or analyzed during the current study available from the corresponding author on reasonable request.

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Figure legends

Figure 1. Overall and recurrence-free survival rate in the discovery cohort

The 5-year survival rate was 71% in the CALLY ≥5 group and 46% in the CALLY <5 group ($p < 0.001$). The 5-year recurrence-free survival rate was 38% in the CALLY ≥5 group and 24% in the CALLY <5 group ($p < 0.001$).

Figure 2. Overall and recurrence-free survival of the discovery cohort classified by CALLY score

The investigation of the CALLY score in the discovery cohort showed that the 5-year survival rate was 69% in the CALLY-S0, 49% in CALLY-S1, 31% in CALLY-S2, and 0% in CALLY-S3 groups ($p < 0.001$). The 5-year recurrence-free survival rate was 38% in the CALLY-S0, 27% in CALLY-S1, 5% in CALLY-S2, and 0% in CALLY-S3 groups ($p < 0.001$).

Figure 3. Comparison of CALLY index with representative biomarkers

The AUC of the CALLY index was 0.666, which was higher than that of the CRP-albumin ratio (0.661), lymphocyte-CRP ratio (0.663), neutrophil-lymphocyte ratio (0.578), platelet-lymphocyte ratio (0.57), and prognostic nutrition index (0.606), suggesting that the accuracy of the CALLY index was higher than that of the other biomarkers.

Figure 4. Overall and recurrence-free survival rates in external validation cohort

The 5-year survival rate in the external validation cohort was 73% in the CALLY ≥5 group and 48% in the CALLY <5 group ($p < 0.001$). The 5-year recurrence-free survival rate was 43% in the CALLY ≥5 group and 26% in the CALLY <5 group ($p < 0.001$).

Figure 5. Overall and recurrence-free survival in the external validation cohort classified by CALLY score

The investigation of CALLY score in the external validation cohort showed that the 5-year survival rate was 72% in the CALLY-S0, 48% in CALLY-S1, and 36% in CALLY-S2 groups ($p < 0.001$). The 5-year recurrence-free survival rate was 41% in the CALLY-S0, 29% in CALLY-S1, and 18% in CALLY-S2 groups ($p < 0.001$); the CALLY-S3 group had only 2 cases.

Figure 6. The 5-year survival rates upon combining the TNM classification and CALLY score

The 5-year survival rate of CALLY-S0 was 74.7%, -S1 was 63.0%, and -S2≥ (S2 and 3) was 37.8% for stage IA+B of the TNM classification. The 5-year survival rate of CALLY-S0 was 68.0%, -S1 was 46.3%, and -S2≥ was 29.9% in for TNM stage II. The 5-year survival rate for CALLY-S0 was 57.7%, -S1 was 24.4%, and -S2≥ was 11.4% for TNM stage III. The 5-year survival rate for CALLY-S0 was

50.0%, and none of patients in the CALLY-S1 and -S2≥ groups survived for 5 years.

Table 1. Comparison between CALLY ≥5 group and CALLY <5 group in discovery cohort

		CALLY ≥5 (n=200)	CALLY <5 (n=184)	p-value
Age (years)		69.8 ± 9.3	69.4 ± 10.8	0.659
Sex (%)	Women	54 (27.0)	34 (18.5)	0.052
	Men	146 (73.0)	150 (81.5)	
Body mass index (Kg/m^2)		23.1 ± 3.3	23.8 ± 3.8	0.045
Diabetes mellitus (%)		50 (25.0)	61 (33.2)	0.091
Alcohol abuse* (%)		24 (12.0)	27 (14.7)	0.456
Positive of HBs-antigen (%)		37 (18.5)	28 (15.2)	0.416
Positive of HCV-antibody (%)		94 (47.0)	56 (30.4)	0.001
White blood count (/μL)		4600 [3700, 5800]	5200 [4370, 6360]	0.004
Lymphocyte count (/μL)		1490 [1198, 1850]	1300 [933, 1673]	<0.001
Platelet count (X10^3/μL)		155 [115, 199]	179 [121, 234]	0.014
Albumin (g/dL)		4.1 [3.8, 4.4]	3.8 [3.5, 4.2]	<0.001
AST (IU/L)		35 [26, 52]	38 [27, 55]	0.359
ALT (IU/L)		33 [22, 52]	30 [19, 47]	0.055
Total bilirubin (mg/dL)		0.7 [0.5, 0.9]	0.7 [0.5, 0.9]	0.758
Prothrombin activity (%)		91 [79, 99]	88 [81, 97]	0.234
C-reactive protein (mg/dL)		0.05 [0.02, 0.09]	0.30 [0.19, 0.81]	<0.001
ICGR15 (%)		13.7 [9.2, 18.8]	13.4 [9.4, 23.1]	0.484
Child-Pugh classification (%)	A	193 (96.5)	163 (88.6)	0.003
	B	7 (3.5)	21 (11.4)	
Maximum tumor size (cm)		3.5 ± 2.6	5.4 ± 4.1	<0.001
Multiple tumor (%)		36 (18.0)	39 (21.2)	0.442
TNM classification** (%)	Ⅰ A+B	90 (45.0)	69 (37.5)	0.003
	Ⅱ	103 (51.5)	88 (47.8)	
	Ⅲ A+B	6 (3.0)	20 (10.9)	
	Ⅳ	1 (0.5)	7 (3.8)	
α-fetoprotein (ng/ml)		9.8 [4.0, 54.5]	14.0 [4.6, 168.1]	0.02
PIVKA-II (mAU/ml)		63 [23, 409]	370 [34, 2426]	<0.001
Anatomical resection (%)		106 (53.0)	111 (60.3)	0.151
Laparoscopic resection (%)		72 (36.0)	54 (29.3)	0.192
Operation time (min)		344 [244, 513]	375 [250, 510]	0.254
Blood loss(ml)		367 [210, 790]	368 [237, 1017]	0.232

Abbreviations: HBs, hepatitis B surface; HCV, hepatitis C virus; AST, aspartate aminotransferase; ALT, alanine aminotransferase; ICGR15, indocyanine green retention rate at 15 minutes; PIVKA-II, protein induced by vitamin K absence or antagonist-II

Age, BMI and maximum tumor size are expressed as mean ± standard deviation. Other data are expressed as median [25th, 75th percentile].

*Alcohol abuse is defined as alcohol consumption of ≥20 g/day
**The 8th edition is used for TNM classification

Table 2. Prognostic factors of overall survival in discovery cohort

		Univariate analysis			Multivariate analysis	
		n	5-y survival rate	p-value	Odds ratio	p-value
Age (years)	<70	186	0.629 (0.553-0.696)	0.104		
	≥70	198	0.561 (0.484-0.631)			
Sex	Women	88	0.678 (0.560-0.771)	0.098		
	Men	296	0.572 (0.511-0.628)			
Body mass index (Kg/m^2)	<25	265	0.582 (0.517-0.642)	0.178		
	≥25	119	0.624 (0.527-0.707)			
Diabetes mellitus	Absent	273	0.609 (0.545-0.667)	0.307		
	Present	111	0.561 (0.460-0.651)			
Alcohol abuse*	Absent	333	0.606 (0.549-0.659)	0.486		
	Present	51	0.528 (0.379-0.657)			
HBs-Antigen	Negative	319	0.567 (0.508-0.622)	0.013	0.55 (0.34-0.89)	0.014
	Positive	65	0.727 (0.598-0.821)			
HCV-Antibody	Negative	234	0.603 (0.535-0.664)	0.556		
	Positive	150	0.582 (0.492-0.661)			
White blood count (/μL)	<4800	189	0.663 (0.587-0.729)	0.059		
	≥4800	195	0.532 (0.456-0.601)			
Platelet count (X10^3/μL)	≥160	194	0.533 (0.457-0.603)	0.089		
	<160	190	0.661 (0.584-0.726)			
AST (IU/L)	<35	184	0.662 (0.586-0.728)	0.061		
	≥35	200	0.534 (0.458-0.604)			
ALT (IU/L)	<30	183	0.604 (0.526-0.674)	0.634		
	≥30	201	0.588 (0.513-0.656)			
ICGR15 (%)	<13	178	0.623 (0.544-0.693)	0.164		
	≥13	206	0.570 (0.496-0.638)			
Child-Pugh classification	A	356	0.609 (0.553-0.659)	0.017	1.59 (0.93-2.69)	0.088
	B	28	0.401 (0.202-0.593)			
TNM classification**	Ⅰ(A, B) or Ⅱ	350	0.622 (0.566-0.673)	<0.001	2.11 (1.36-3.26)	<0.001
	Ⅲ(A, B) or Ⅳ	34	0.333 (0.180-0.495)			
α-fetoprotein (ng/ml)	<30	248	0.677 (0.611-0.733)	<0.001	1.76 (1.27-2.44)	<0.001
	≥30	136	0.445 (0.355-0.530)			
PIVKA-Ⅱ (mAU/ml)	<100	181	0.737 (0.662-0.798)	<0.001	1.98 (1.39-2.80)	<0.001
	≥100	203	0.471 (0.398-0.541)			
Anatomic resection	No	167	0.650 (0.568-0.721)	0.053		
	Yes	217	0.555 (0.483-0.621)			
Laparoscopic resection	No	258	0.572 (0.507-0.633)	0.051		
	Yes	126	0.642 (0.546-0.723)			
Operation time (min)	<480	275	0.615 (0.552-0.672)	0.127		
	≥480	109	0.545 (0.442-0.637)			
Blood loss (ml)	<500	225	0.662 (0.587-0.727)	0.009	1.33 (0.97-1.84)	0.078
	≥500	159	0.529 (0.452-0.600)			
CALLY index	≥5	200	0.710 (0.640-0.770)	<0.001	1.70 (1.21-2.38)	0.002
	<5	184	0.460 (0.380-0.530)			

Abbreviations: HBs, hepatitis B surface; HCV, hepatitis C virus; AST, aspartate aminotransferase; ALT, alanine aminotransferase; ICGR15, indocyanine green retention rate at 15 minutes; PIVKA-II, protein induced by vitamin K absence or antagonist-II; CALLY index, CRP-Albumin-Lymphocyte index

*Alcohol abuse is defined as alcohol consumption of ≥20 g/day
**The 8th edition is used for TNM classification

Table 3. Prognostic factors of recurrence free survival in discovery cohort

		Univariate analysis			Multivariate analysis	
		n	5-y survival rate	p-value	Odds ratio	p-value
Age (years)	<70	186	0.357 (0.285-0.429)	0.545		
	≥70	198	0.279 (0.211-0.351)			
Sex	Women	88	0.428 (0.315-0.537)	0.1		
	Men	296	0.290 (0.235-0.346)			
Body mass index (Kg/m^2)	<25	265	0.321 (0.261-0.382)	0.899		
	≥25	119	0.316 (0.228-0.407)			
Diabetes mellitus	Absent	273	0.323 (0.264-0.383)	0.991		
	Present	111	0.306 (0.214-0.402)			
Alcohol abuse*	Absent	333	0.315 (0.262-0.369)	0.499		
	Present	51	0.347 (0.207-0.491)			
HBs-Antigen	Negative	319	0.311 (0.256-0.367)	0.24		
	Positive	65	0.362 (0.245-0.480)			
HCV-Antibody	Negative	234	0.344 (0.280-0.409)	0.406		
	Positive	150	0.277 (0.200-0.358)			
White blood count (/μL)	<4800	189	0.364 (0.290-0.437)	0.07		
	≥4800	195	0.277 (0.211-0.346)			
Platelet count (X10^3/μL)	≥160	194	0.307 (0.238-0.378)	0.325		
	<160	190	0.332 (0.261-0.405)			
AST (IU/L)	<35	184	0.362 (0.287-0.438)	0.009	1.21 (0.93-1.56)	0.16
	≥35	200	0.280 (0.216-0.348)			
ALT (IU/L)	<30	183	0.344 (0.269-0.420)	0.17		
	≥30	201	0.297 (0.232-0.366)			
ICGR15 (%)	<13	178	0.358 (0.283-0.434)	0.099		
	≥13	206	0.284 (0.219-0.353)			
Child-Pugh classification	A	356	0.331 (0.279-0.384)	0.029	1.44 (0.91-2.26)	0.12
	B	28	0.172 (0.056-0.341)			
TNM classification**	Ⅰ(A, B) or Ⅱ	350	0.342 (0.289-0.396)	<0.001	2.24 (1.48-3.41)	<0.001
	Ⅲ(A, B) or Ⅳ	34	NA (NA- NA)			
α-fetoprotein (ng/ml)	<30	248	0.343 (0.280-0.406)	0.002	1.35 (1.03-1.76)	0.029
	≥30	136	0.280 (0.201-0.365)			
PIVKA-Ⅱ(mAU/ml)	<100	181	0.401 (0.325-0.476)	<0.001	1.46 (1.12-1.89)	0.004
	≥100	203	0.243 (0.180-0.311)			
Anatomic resection	No	167	0.338 (0.262-0.415)	0.246		
	Yes	217	0.305 (0.240-0.372)			
Laparoscopic resection	No	258	0.299 (0.239-0.360)	0.064		
	Yes	126	0.361 (0.271-0.451)			
Operation time (min)	<480	275	0.314 (0.256-0.373)	0.973		
	≥480	109	0.334 (0.240-0.431)			
Blood loss (ml)	<500	225	0.356 (0.290-0.424)	0.032	1.13 (0.87-1.46)	0.37
	≥500	159	0.266 (0.194-0.343)			
CALLY index	≥5	200	0.389 (0.319-0.459)	<0.001	1.41 (1.08-1.83)	0.011
	<5	184	0.231 (0.165-0.305)			

Abbreviations: HBs, hepatitis B surface; HCV, hepatitis C virus; AST, aspartate aminotransferase; ALT, alanine aminotransferase; ICGR15, indocyanine green retention rate at 15 minutes; PIVKA-II, protein induced by vitamin K absence or antagonist-II; CALLY index, CRP-Albumin-Lymphocyte index

*Alcohol abuse is defined as alcohol consumption of ≥20 g/day
**The 8th edition is used for TNM classification

Table 4. Comparison between discovery cohort and external validation cohort

		Discovery (n=384)	Validation (n=267)	p-value
Age (years)		69.6 ± 10.1	68.3 ± 9.7	0.1
Sex (%)	Women	88 (22.9)	65 (24.3)	0.707
	Men	296 (77.1)	202 (75.7)	
Body mass index (Kg/m^2)		23.4 ± 3.6	23.2 ± 3.8	0.5
Diabetes mellitus (%)		111 (28.9)	99 (37.1)	0.033
Alcohol abuse* (%)		51 (13.3)	35 (13.1)	>0.999
Positive of HBs-antigen (%)		65 (16.9)	49 (18.4)	0.675
Positive of HCV-antibody (%)		150 (39.1)	134 (50.2)	0.005
White blood count (/μL)		4975 [3900, 6177]	5000 [4000, 6100]	0.785
Lymphocyte count (/μL)		1400 [1099, 1800]	1426 [1100, 1866]	0.387
Platelet count (X10^3/μL)		161 [116, 217]	140 [108, 180]	<0.001
Albumin (g/dL)		4.0 [3.6, 4.3]	4.1 [3.8, 4.4]	0.01
AST (IU/L)		37 [26, 53]	37 [28, 57]	0.235
ALT (IU/L)		31 [20, 50]	31 [21, 50]	0.603
Total bilirubin (mg/dL)		0.7 [0.5, 0.9]	0.7 [0.5, 0.9]	0.152
Prothrombin activity (%)		89 [80, 98]	90 [82, 100]	0.079
C-reactive protein (mg/dL)		0.10 [0.04, 0.30]	0.08 [0.03, 0.26]	0.172
ICGR15 (%)		13.6 [9.2, 20.9]	14.0 [9.3, 19.0]	0.787
Child-Pugh classification (%)	A	356 (92.7)	256 (95.9)	0.13
	B	28 (7.3)	11 (4.1)	
Maximum tumor size (cm)		4.4 ± 3.5	4.6 ± 3.8	0.596
Multiple tumor (%)		75 (19.5)	55 (20.6)	0.765
TNM classification** (%)	Ⅰ A+B	159 (41.4)	169 (63.3)	<0.001
	Ⅱ	191 (49.7)	49 (18.4)	
	ⅢA+B	26 (6.8)	44 (16.5)	
	Ⅳ	8 (2.1)	5 (1.9)	
α-fetoprotein (ng/ml)		11.7 [4.2, 88.3]	10.9 [4.8, 92.9]	0.562
PIVKA-II (mAU/ml)		136 [26, 1040]	130 [30, 1222]	0.692
Anatomical resection (%)		217 (56.5)	158 (59.2)	0.52
Laparoscopic resection (%)		126 (32.8)	73 (27.3)	0.142
Operation time (min)		363 [246, 513]	274 [212, 345]	<0.001
Blood loss(ml)		368 [228, 886]	415 [136, 1134]	0.635

Abbreviations: HBs, hepatitis B surface; HCV, hepatitis C virus; AST, aspartate aminotransferase; ALT, alanine aminotransferase; ICGR15, indocyanine green retention rate at 15 minutes; PIVKA-II, protein induced by vitamin K absence or antagonist-II

Age, BMI and maximum tumor size are expressed as mean ± standard deviation. Other data are expressed as median [25th, 75th percentile].

*Alcohol abuse is defined as alcohol consumption of ≥20 g/day
**The 8th edition is used for TNM classification

Table 5. Prognostic factors of overall survival in external validation cohort

		Univariate analysis			Multivariate analysis	
		n	5-y survival rate	p-value	Odds ratio	p-value
Age (years)	<70	127	0.666 (0.574-0.743)	0.294		
	≥70	140	0.606 (0.513-0.686)			
Sex	Women	65	0.678 (0.536-0.784)	0.372		
	Men	202	0.623 (0.550-0.688)			
Body mass index (Kg/m^2)	<25	189	0.627 (0.550-0.696)	0.93		
	≥25	78	0.654 (0.535-0.750)			
Diabetes mellitus	Absent	168	0.642 (0.560-0.713)	0.928		
	Present	99	0.623 (0.516-0.714)			
Alcohol abuse*	Absent	232	0.644 (0.575-0.705)	0.433		
	Present	35	0.580 (0.394-0.728)			
HBs-Antigen	Negative	218	0.623 (0.551-0.687)	0.223		
	Positive	49	0.683 (0.530-0.796)			
HCV-Antibody	Negative	133	0.623 (0.532-0.701)	0.774		
	Positive	134	0.649 (0.554-0.728)			
White blood count (/μL)	<5000	131	0.624 (0.530-0.705)	0.927		
	≥5000	136	0.646 (0.556-0.723)			
Platelet count (X10^3/μL)	≥150	122	0.655 (0.562-0.733)	0.669		
	<150	145	0.616 (0.524-0.695)			
AST (IU/L)	<35	117	0.689 (0.592-0.767)	0.101		
	≥35	150	0.593 (0.504-0.670)			
ALT (IU/L)	<30	120	0.580 (0.481-0.667)	0.077		
	≥30	147	0.679 (0.593-0.750)			
ICGR15 (%)	<14	127	0.668 (0.576-0.745)	0.109		
	≥14	140	0.603 (0.511-0.684)			
Child-Pugh classification	A	256	0.644 (0.579-0.702)	0.026	2.02 (0.91-4.48)	0.082
	B	11	0.436 (0.147-0.699)			
TNM classification**	Ⅰ(A, B) or Ⅱ	218	0.689 (0.619-0.749)	<0.001	1.85 (1.18-2.91)	0.007
	Ⅲ(A, B) or Ⅳ	49	0.396 (0.255-0.534)			
α-fetoprotein (ng/ml)	<30	173	0.719 (0.643-0.783)	<0.001	1.49 (0.99-2.23)	0.053
	≥30	94	0.477 (0.366-0.579)			
PIVKA-Ⅱ(mAU/ml)	<100	120	0.759 (0.665-0.829)	0.001	1.35 (0.88-2.07)	0.16
	≥100	147	0.537 (0.449-0.616)			
Anatomic resection	No	109	0.669 (0.562-0.756)	0.266		
	Yes	158	0.613 (0.531-0.686)			
Laparoscopic resection	No	194	0.611 (0.535-0.678)	0.039	0.88 (0.53-1.47)	0.63
	Yes	73	0.704 (0.575-0.801)			
Operation time (min)	<300	160	0.691 (0.605-0.761)	0.046	1.03 (0.69-1.53)	0.89
	≥300	107	0.558 (0.459-0.647)			
Blood loss (ml)	<500	143	0.715 (0.627-0.786)	0.002	1.45 (0.96-2.21)	0.08
	≥500	124	0.548 (0.453-0.632)			
CALLY index	≥5	162	0.737 (0.656-0.801)	<0.001	1.81 (1.21-2.71)	0.003
	<5	105	0.486 (0.385-0.580)			

Abbreviations: HBs, hepatitis B surface; HCV, hepatitis C virus; AST, aspartate aminotransferase; ALT, alanine aminotransferase; ICGR15, indocyanine green retention rate at 15 minutes; PIVKA-II, protein induced by vitamin K absence or antagonist-II; CALLY index, CRP-Albumin-Lymphocyte index

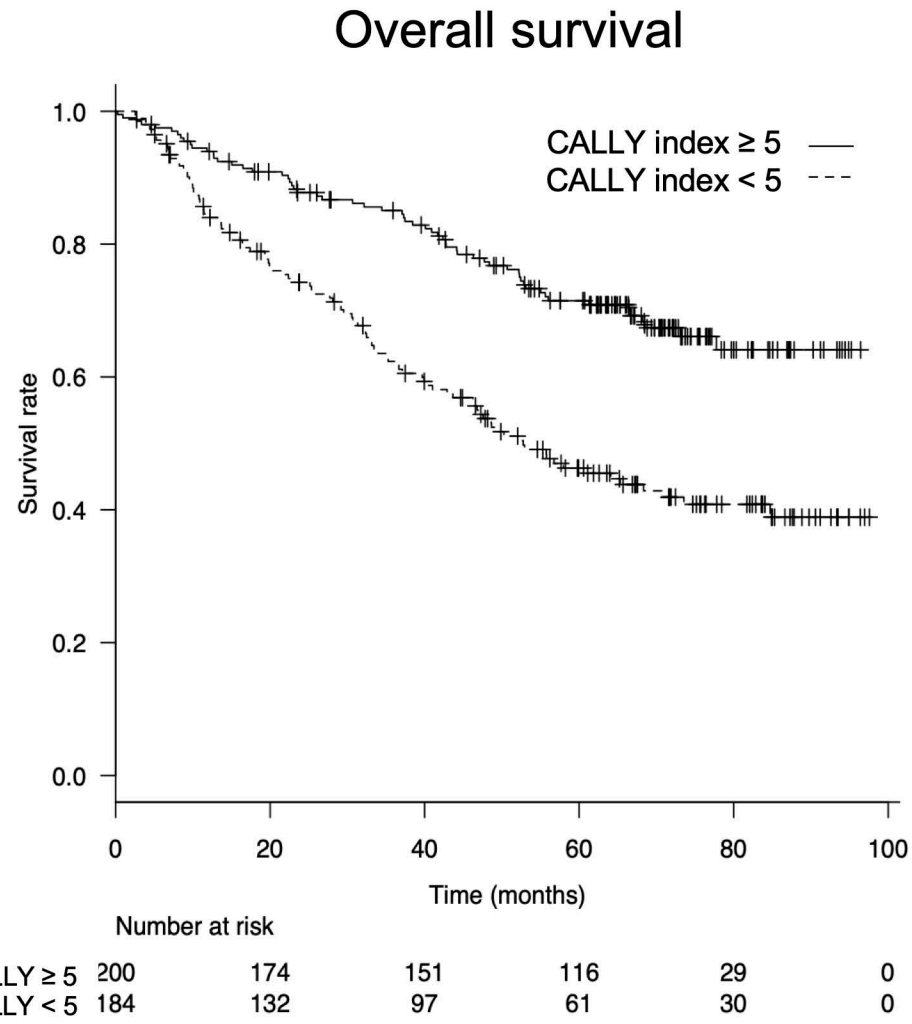
*Alcohol abuse is defined as alcohol consumption of ≥20 g/day
**The 8th edition is used for TNM classification

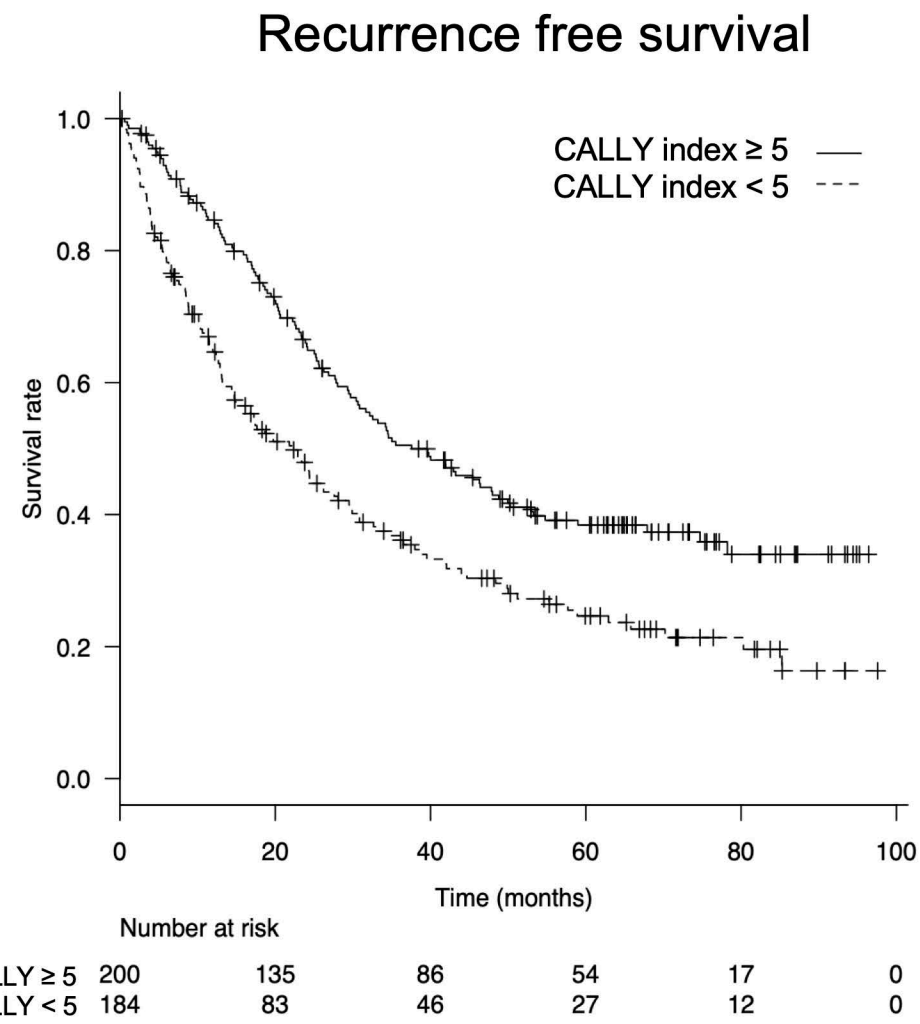
Table 6. Prognostic factors of recurrence free survival in external validation cohort

		Univariate analysis			Multivariate analysis	
		n	5-y survival rate	p-value	Odds ratio	p-value
Age (years)	<70	127	0.318 (0.234-0.404)	0.104		
	≥70	140	0.418 (0.325-0.509)			
Sex	Women	65	0.352 (0.227-0.480)	0.699		
	Men	202	0.372 (0.299-0.444)			
Body mass index (Kg/m^2)	<25	189	0.395 (0.317-0.472)	0.108		
	≥25	78	0.309 (0.206-0.417)			
Diabetes mellitus	Absent	168	0.339 (0.260-0.420)	0.47		
	Present	99	0.406 (0.303-0.506)			
Alcohol abuse*	Absent	232	0.386 (0.318-0.454)	0.262		
	Present	35	0.245 (0.107-0.414)			
HBs-Antigen	Negative	218	0.366 (0.297-0.436)	0.778		
	Positive	49	0.371 (0.227-0.515)			
HCV-Antibody	Negative	133	0.335 (0.251-0.422)	0.235		
	Positive	134	0.402 (0.309-0.492)			
White blood count (/μL)	<5000	131	0.287 (0.204-0.375)	0.032	0.65 (0.47-0.90)	0.009
	≥5000	136	0.446 (0.354-0.533)			
Platelet count (X10^3/μL)	≥150	122	0.400 (0.307-0.491)	0.489		
	<150	145	0.336 (0.251-0.423)			
AST (IU/L)	<35	117	0.440 (0.341-0.533)	0.01	1.21 (0.86-1.69)	0.28
	≥35	150	0.310 (0.229-0.393)			
ALT (IU/L)	<30	120	0.406 (0.308-0.501)	0.398		
	≥30	147	0.338 (0.257-0.422)			
ICGR15 (%)	<14	127	0.399 (0.309-0.488)	0.48		
	≥14	140	0.330 (0.243-0.420)			
Child-Pugh classification	A	256	0.370 (0.305-0.435)	0.241		
	B	11	0.312 (0.075-0.591)			
TNM classification**	Ⅰ(A, B) or Ⅱ	218	0.414 (0.343-0.485)	<0.001	1.89 (1.28-2.77)	0.001
	Ⅲ(A, B) or Ⅳ	49	0.151 (0.059-0.282)			
α-fetoprotein (ng/ml)	<30	173	0.411 (0.331-0.489)	0.004	1.40 (0.99-1.97)	0.054
	≥30	94	0.289 (0.190-0.394)			
PIVKA-Ⅱ(mAU/ml)	<100	120	0.382 (0.285-0.477)	0.175		
	≥100	147	0.354 (0.272-0.437)			
Anatomic resection	No	109	0.334 (0.234-0.436)	0.946		
	Yes	158	0.384 (0.303-0.464)			
Laparoscopic resection	No	194	0.359 (0.286-0.433)	0.345		
	Yes	73	0.390 (0.268-0.509)			
Operation time (min)	<300	160	0.388 (0.302-0.473)	0.053		
	≥300	107	0.329 (0.238-0.423)			
Blood loss (ml)	<500	143	0.429 (0.339-0.515)	0.005	1.55 (1.12-2.16)	0.009
	≥500	124	0.292 (0.208-0.382)			
CALLY index	≥5	162	0.433 (0.349-0.514)	<0.001	1.56 (1.12-2.15)	0.007
	<5	105	0.265 (0.177-0.362)			

Abbreviations: HBs, hepatitis B surface; HCV, hepatitis C virus; AST, aspartate aminotransferase; ALT, alanine aminotransferase; ICGR15, indocyanine green retention rate at 15 minutes; PIVKA-II, protein induced by vitamin K absence or antagonist-II; CALLY index, CRP-Albumin-Lymphocyte index

*Alcohol abuse is defined as alcohol consumption of ≥20 g/day
**The 8th edition is used for TNM classification

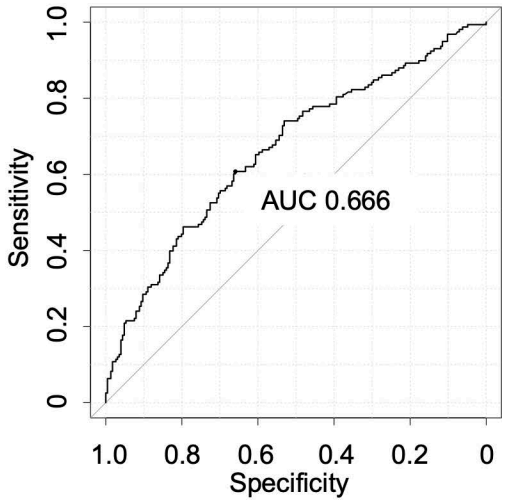




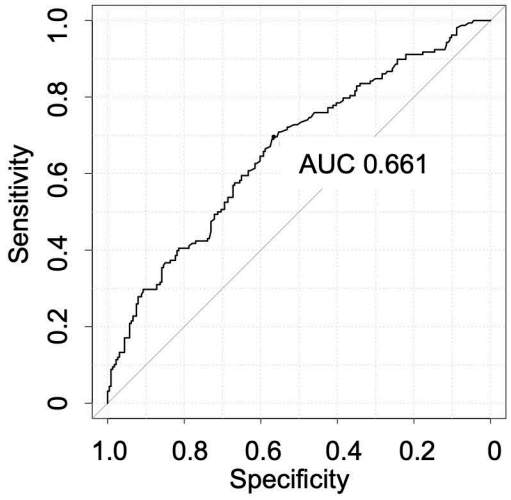
		n	5-y survival rate	Median survival	p-value
CALLY	≥ 5	200	0.71 (0.64-0.77)	NA (NA-NA)	<0.001
	< 5	184	0.46 (0.38-0.53)	52.7 (42.9-70.8)	

		n	5-y survival rate	Median survival	p-value
CALLY	≥ 5	200	0.38 (0.31-0.45)	37.5 (30.4-47.9)	<0.001
	< 5	184	0.24 (0.18-0.31)	22.2 (14.7-28.0)	

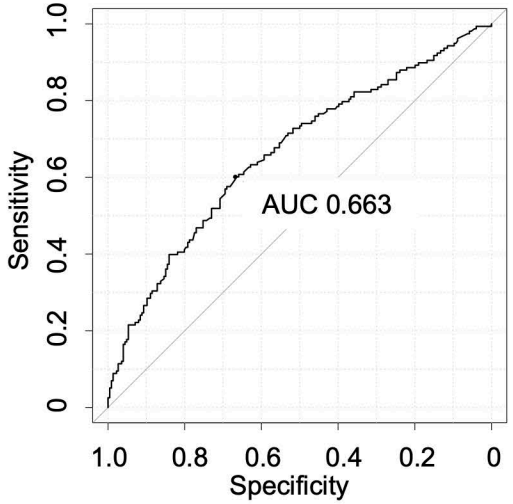
Figure 1



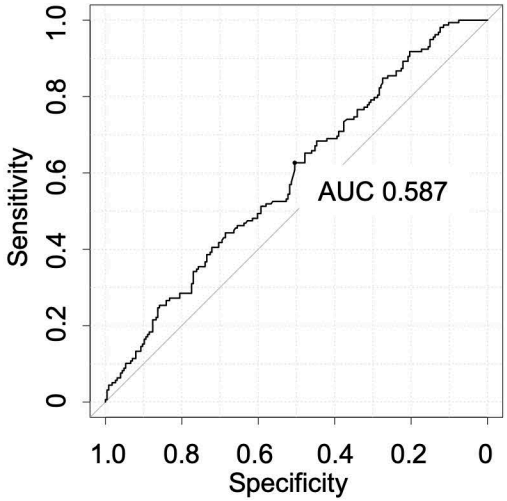
CALLY index (CRP-Albumin-Lymphocyte index)



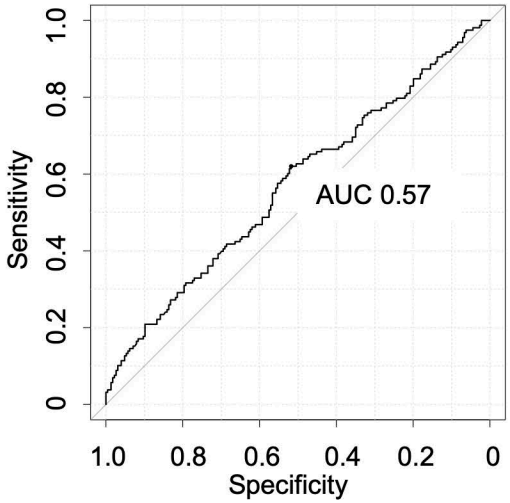
CAR (CRP-Albumin ratio)



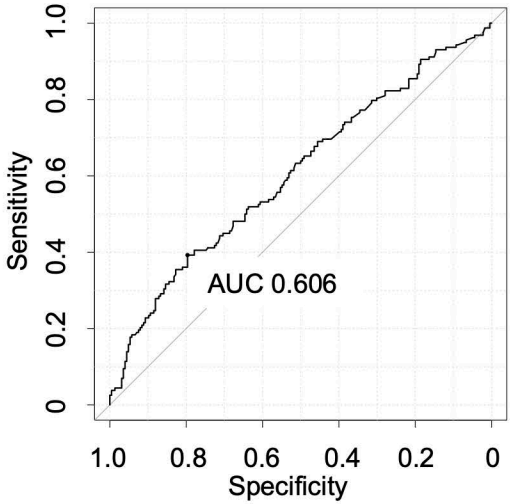
LCR (Lymphocyte-CRP ratio)



NLR (Neutrophil-Lymphocyte ratio)



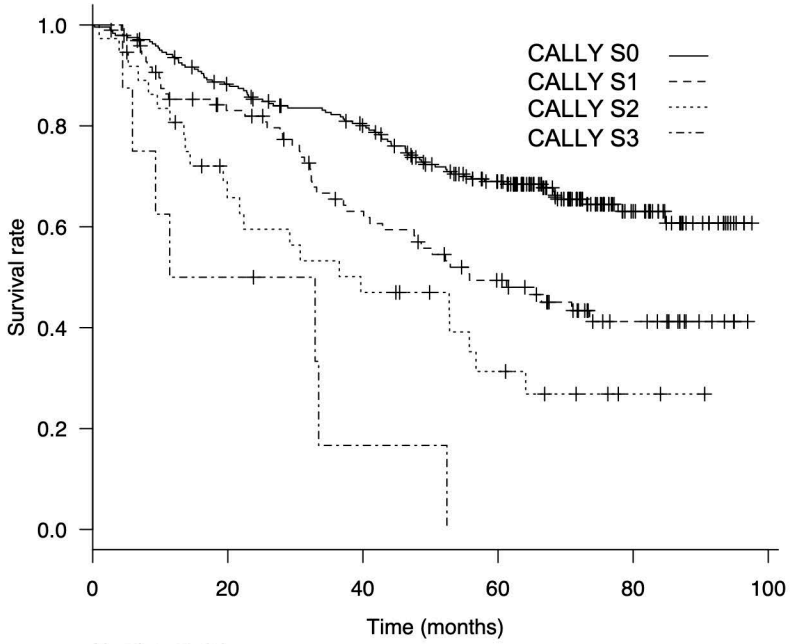
PLR (Platelet-Lymphocyte ratio)



PNI (Prognosis nutrition index)

Figure 2

Overall survival

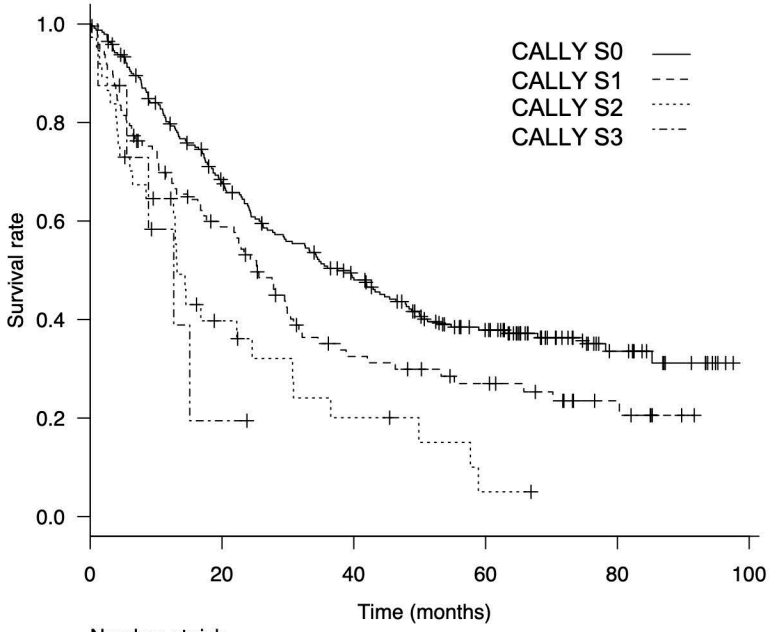


Number at risk

CALLY S0	242	207	180	132	41	0
CALLY S1	97	74	52	37	16	0
CALLY S2	37	21	15	8	2	0
CALLY S3	8	4	1	0	0	0

		n	5-y survival rate	Median survival	p-value
CALLY	S0	242	0.690 (0.625-0.745)	NA (NA-NA)	<0.001
	S1	97	0.494 (0.384-0.595)	55.7 (41.0-NA)	
	S2	37	0.313 (0.158-0.482)	39.6 (19.9-56.7)	
	S3	8	NA NA	22.1 (4.43-NA)	

Recurrence free survival



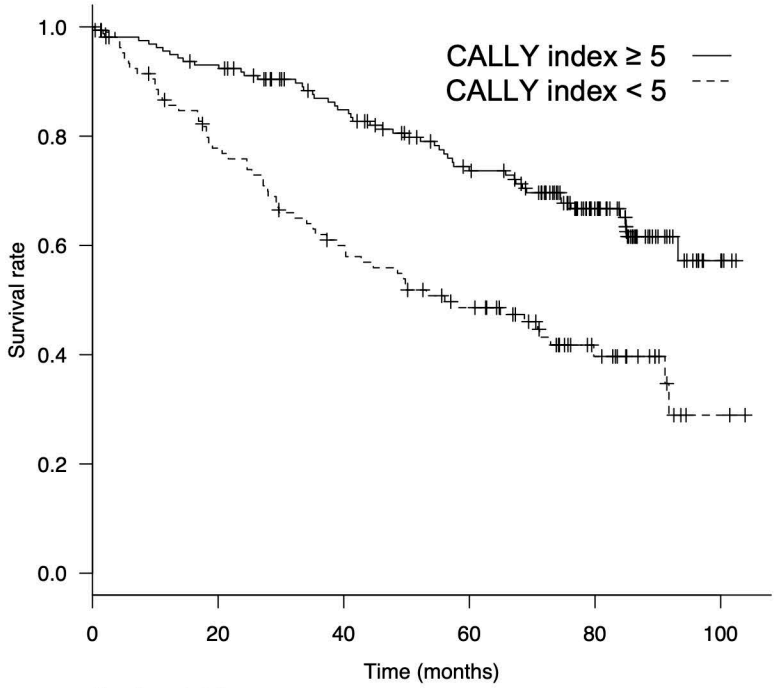
Number at risk

CALLY S0	242	154	102	62	21	0
CALLY S1	97	52	25	18	8	0
CALLY S2	37	11	5	1	0	0
CALLY S3	8	1	0	0	0	0

		n	5-y survival rate	Median survival	p-value
CALLY	S0	242	0.379 (0.314-0.443)	37.5 (29.4-47.8)	<0.001
	S1	97	0.270 (0.179-0.369)	25.3 (17.6-30.8)	
	S2	37	0.050 (0.004-0.200)	13.1 (6.43-24.5)	
	S3	8	NA NA	12.6 (1.18-NA)	

Figure 3

Overall survival

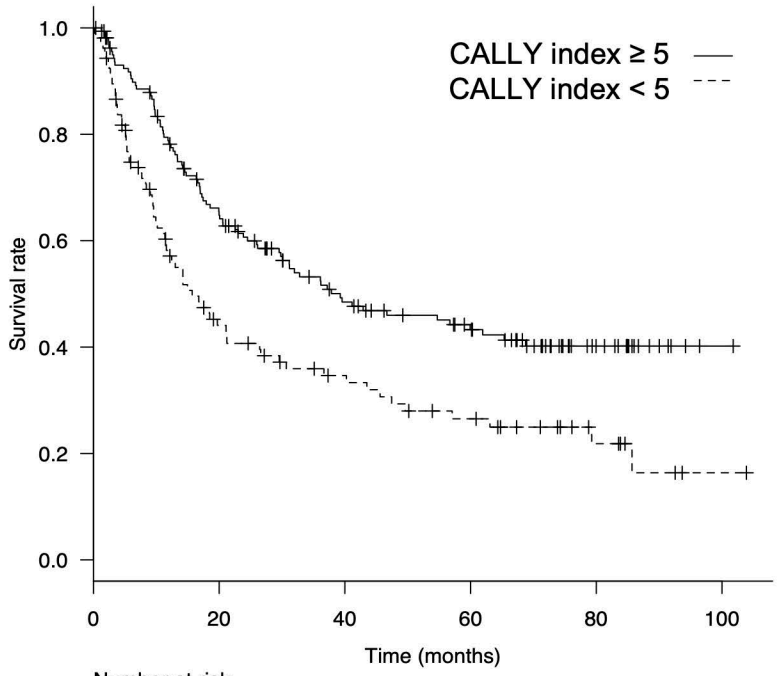


Number at risk

CALLY ≥ 5	162	145	121	95	52	5
CALLY < 5	105	79	59	44	19	2

		n	5-y survival rate	Median survival	p-value
CALLY	≥ 5	162	0.737 (0.656-0.801)	NA (93.2-NA)	<0.001
	< 5	105	0.486 (0.385-0.580)	56.0 (39.0-79.8)	

Recurrence free survival



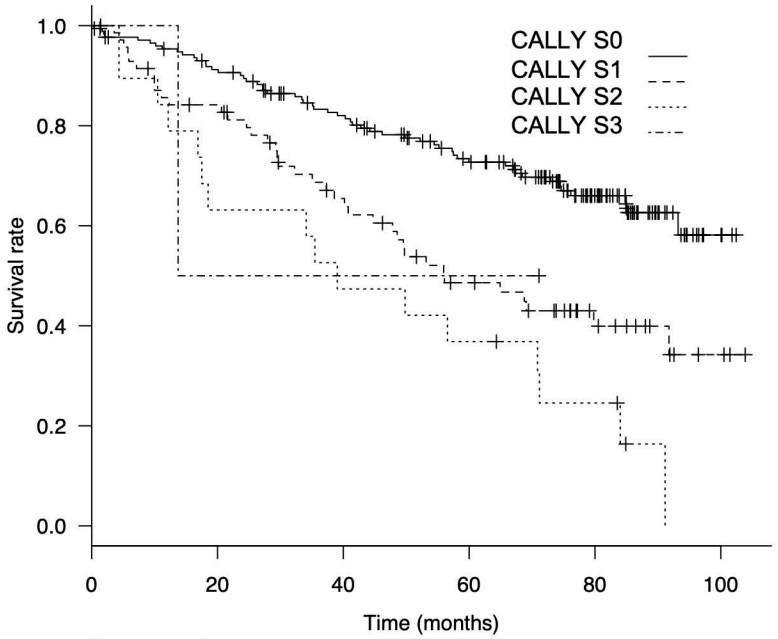
Number at risk

CALLY ≥ 5	162	96	61	46	18	1
CALLY < 5	105	39	26	18	7	1

		n	5-y survival rate	Median survival	p-value
CALLY	≥ 5	162	0.433 (0.349-0.514)	39.2 (29.5-65.3)	<0.001
	< 5	105	0.265 (0.177-0.362)	15.7 (11.4-26.4)	

Figure 4

Overall survival

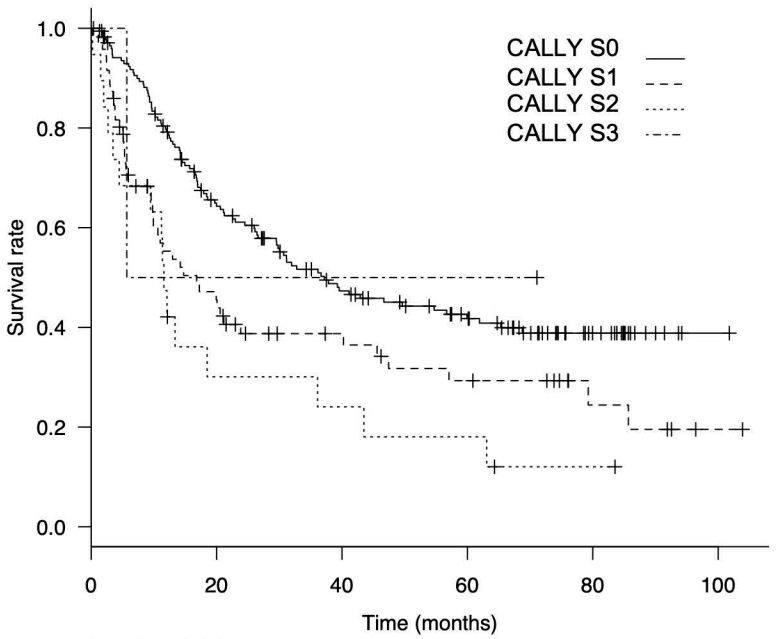


Number at risk

CALLY S0	175	154	130	104	54	4
CALLY S1	71	57	40	27	13	3
CALLY S2	19	12	9	7	4	0
CALLY S3	2	1	1	1	0	0

		n	5-y survival rate	Median survival	p-value
CALLY	S0	175	0.727 (0.651-0.790)	NA (93.2-NA)	<0.001
	S1	71	0.486 (0.358-0.603)	56.0 (40.7-91.7)	
	S2	19	0.368 (0.165-0.575)	39.0 (16.8-71.1)	
	S3	2	0.500 (0.006-0.910)	13.7 (13.7-NA)	

Recurrence free survival



Number at risk

CALLY S0	175	101	65	48	19	1
CALLY S1	71	28	17	12	5	1
CALLY S2	19	5	4	3	1	0
CALLY S3	2	1	1	1	0	0

		n	5-y survival rate	Median survival	p-value
CALLY	S0	175	0.418 (0.337-0.496)	37.1 (26.6-59.9)	<0.001
	S1	71	0.293 (0.179-0.417)	16.8 (9.82-40.2)	
	S2	19	0.180 (0.046-0.386)	11.5 (3.48-36.1)	
	S3	2	0.500 (0.006-0.910)	5.68 (5.68-NA)	

Figure 5

All cohort (n=651)				
Combination of TNM classification and CALLY score		CALLY score		
		S2≥ (n=66)	S1 (n=168)	S0 (n=417)
TNM classification	IA+B (n=328)	37.8 (n=28)	63.0 (n=78)	74.7 (n=222)
	II (n=240)	29.9 (n=24)	46.3 (n=60)	68.0 (n=156)
	IIIA+B (n=70)	11.4 (n=11)	24.4 (n=26)	57.7 (n=33)
	IV (n=13)	0 (n=3)	0 (n=4)	50.0 (n=6)
5-year survival rate				
>60%				
20-60%				
<20%				

Figure 6