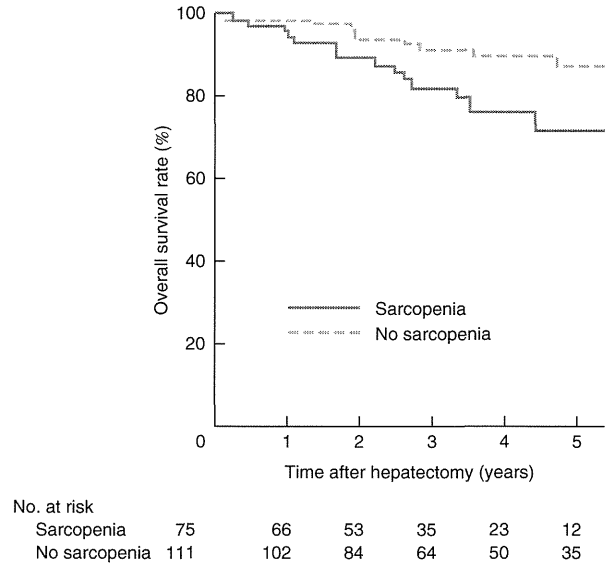
## Statistical analysis

Associations of continuous and categorical variables with relevant outcome variables were assessed using the Mann–Whitney *U* test and Fisher's exact test respectively. The variable skeletal muscle was not *a priori* categorized into a binary variable (sarcopenia present or not), because categorizing a continuous predictor would result in an inevitable loss of information. Instead, the multivariable fractional polynomial (MFP) approach was adopted. In the polynomial fractional model, for each continuous variable $X$, one or two terms of the form $X^p$ were fitted with powers, $p$, chosen from ($-2$, $-1$, $-0{\cdot}5$, 0, 0·5, 1, 2 and 3). The results of the MFP analysis revealed that the most appropriate power for skeletal muscle mass in the MFP model was given in the form of $X$ (that is, $p = 1$), allowing expression of a final multivariable model in terms of the usual Cox regression model. Therefore, the results of the usual Cox model are reported here, giving the results of the log rank tests for the association between the presence of sarcopenia (as defined by dichotomizing skeletal muscle mass) and overall or disease-free survival[23]. To identify prognostic factors after hepatectomy, all variables were included in the overall multivariable Cox proportional model in the analyses of both overall and recurrence-free survival using the backward selection method. The overall and recurrence-free survival curves were analysed by the Kaplan-Meier method and compared with the log rank test. All analyses were performed with StatView® 5.0 software (Abacus Concepts, Berkeley, California, USA). $P < 0{\cdot}050$ was considered statistically significant.

## Results

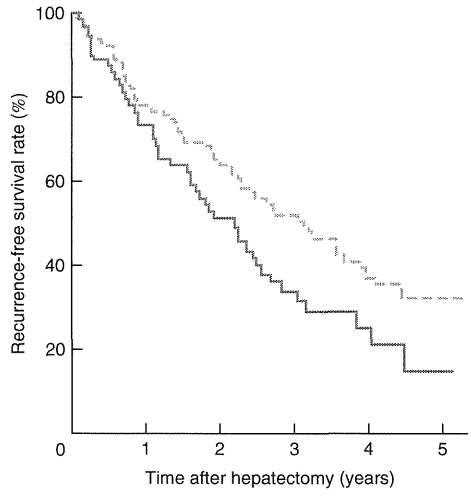
In total, 186 patients with HCC were identified from the database, of whom 75 (40·3 per cent; 50 men and 25 women) had sarcopenia. Clinicopathological characteristics of patients with and without sarcopenia are shown in *Table 1*. Women were more likely to have sarcopenia than men. Patients with sarcopenia had a significantly lower BMI than those without. Regarding liver function, serum albumin levels were significantly lower and ICGR15 values were significantly higher in patients with sarcopenia than in those without. Other host-related factors such as age, hepatitis, diabetes mellitus, Child–Pugh grade, MELD score and liver cirrhosis were not related to the presence of sarcopenia. There were no significant differences in tumour-related factors or surgical outcomes between the two groups. Operative details are shown in *Table S1* (supporting information).




No. at risk

| | | | | | | |
|---|---|---|---|---|---|---|
| Sarcopenia | 75 | 66 | 53 | 35 | 23 | 12 |
| No sarcopenia | 111 | 102 | 84 | 64 | 50 | 35 |

**a** Overall survival




No. at risk

| | | | | | | |
|---|---|---|---|---|---|---|
| Sarcopenia | 75 | 45 | 30 | 14 | 7 | 2 |
| No sarcopenia | 111 | 80 | 61 | 40 | 22 | 12 |

**b** Recurrence-free survival

**Fig. 2** **a** Overall and **b** recurrence-free survival curves after liver resection in patients with, and without sarcopenia. **a** $P = 0{\cdot}001$, **b** $P = 0{\cdot}013$ (log rank test)

Overall and recurrence-free survival curves for patients with and without sarcopenia are shown in *Fig. 2*. Overall and recurrence-free 5-year survival rates were 71 and 13 per cent respectively in patients with sarcopenia, and 83·7 and 33·2 per cent in patients without sarcopenia (*Fig. 2*). Patients with sarcopenia had a significantly worse prognosis

**Table 2** Univariable and multivariable analysis of clinicopathological factors and overall survival following partial hepatectomy with curative intent for hepatocellular carcinoma

| | Univariable analysis | | Multivariable analysis | |
|---|---|---|---|---|
| | Hazard ratio | *P** | Hazard ratio | *P*† |
| Age | 1·02 (0·98, 1·07) | 0·323 | | |
| Female sex | 1·17 (0·42, 2·79) | 0·746 | | |
| Skeletal muscle mass | 0·92 (0·86, 0·97) | 0·004 | 0·90 (0·84, 0·96) | 0·002 |
| Body mass index | 0·92 (0·81, 1·04) | 0·199 | | |
| Albumin | 0·47 (0·21, 1·14) | 0·092 | | |
| ICGR15 | 1·02 (0·97, 1·07) | 0·512 | | |
| MELD score | 1·08 (0·86, 1·25) | 0·460 | | |
| Liver fibrosis + cirrhosis | 3·97 (1·50, 13·67) | 0·004 | | |
| Tumour size | 1·10 (0·98, 1·22) | 0·906 | | |
| Multiple tumours | 1·60 (0·65, 3·64) | 0·292 | | |
| TNM stage III + IV | 1·62 (0·70, 3·62) | 0·255 | | |
| Poor differentiation | 2·26 (0·98, 5·16) | 0·055 | 2·47 (1·05, 5·81) | 0·021 |
| Microvascular invasion | 2·39 (1·05, 5·41) | 0·038 | 3·21 (1·29, 7·94) | 0·018 |
| Intrahepatic metastases | 1·67 (0·55, 4·15) | 0·333 | | |
| α-Fetoprotein | 1·00 (1·00, 1·00) | 0·335 | | |
| DCP | 1·00 (1·00, 1·00) | 0·267 | | |
| Postoperative complications | 2·76 (1·23, 6·28) | 0·014 | 3·27 (1·39, 7·69) | 0·007 |

Values in parentheses are 95 per cent confidence intervals. ICGR15, indocyanine green dye retention test at 15 min; MELD, Model for End-Stage Liver Disease; TNM, tumour node metastasis; DCP, des-γ-carboxyprothrombin. *Log rank test; †Cox proportional model.

**Table 3** Univariable and multivariable analysis of clinicopathological factors and recurrence-free survival following partial hepatectomy with curative intent for hepatocellular carcinoma

| | Univariable analysis | | Multivariable analysis | |
|---|---|---|---|---|
| | Hazard ratio | *P** | Hazard ratio | *P*† |
| Age | 1·01 (1·00, 1·04) | 0·139 | | |
| Female sex | 1·02 (0·63, 1·59) | 0·918 | | |
| Skeletal muscle mass | 0·98 (0·95, 1·00) | 0·049 | 0·97 (0·95, 1·00) | 0·016 |
| Body mass index | 0·94 (0·88, 1·02) | 0·076 | | |
| Albumin | 0·49 (0·33, 0·75) | 0·001 | | |
| ICGR15 | 1·03 (1·01, 1·06) | 0·048 | 1·02 (1·02, 1·07) | 0·001 |
| MELD score | 1·03 (0·93, 1·12) | 0·526 | | |
| Liver fibrosis + cirrhosis | 1·98 (1·32, 3·01) | 0·001 | | |
| Tumour size | 1·00 (0·98, 1·11) | 0·141 | | |
| Multiple tumours | 1·89 (1·22, 2·84) | 0·005 | | |
| TNM stage III + IV | 2·44 (1·64, 3·61) | 0·001 | 2·13 (1·38, 3·29) | 0·001 |
| Poor differentiation | 1·58 (1·04, 2·35) | 0·033 | | |
| Microvascular invasion | 2·39 (1·05, 5·41) | 0·038 | | |
| Intrahepatic metastases | 2·14 (1·30, 3·38) | 0·003 | 2·37 (1·38, 4·06) | 0·018 |
| α-Fetoprotein | 1·00 (1·00, 1·00) | 0·001 | | |
| DCP | 1·00 (1·00, 1·00) | 0·006 | 1·00 (1·00, 1·00) | 0·001 |
| Postoperative complications | 1·11 (0·73, 1·67) | 0·617 | | |

Values in parentheses are 95 per cent confidence intervals. ICGR15, indocyanine green dye retention test at 15 min; MELD, Model for End-Stage Liver Disease; TNM, tumour node metastasis; DCP, des-γ-carboxyprothrombin. *Log rank test; †Cox proportional model.

than those without in terms of both overall ($P = 0·001$) and recurrence-free survival ($P = 0·013$).

In univariable analysis, significant prognostic factors for overall survival were low skeletal muscle mass, and presence of liver cirrhosis, MVI and postoperative complications (*Table 2*). Significant prognostic factors for recurrence-free survival were lower skeletal muscle mass, serum albumin level, liver cirrhosis, tumour number, tumour stage, poorly differentiated HCC, MVI, intrahepatic metastases, and serum AFP and DCP levels (*Table 3*). Multivariable analysis identified four poor prognostic factors (low skeletal muscle mass, poorly differentiated HCC, MVI and postoperative complications) that influenced overall survival, and five poor prognostic factors (low skeletal muscle mass, high

ICGR15 value, high serum DCP level, presence of intrahepatic metastases, and stage III + IV disease) that influenced recurrence-free survival (*Tables 2* and *3*).

## Discussion

The findings of this retrospective single-centre study suggest that sarcopenia is an independent prognostic factor for overall and recurrence-free survival in patients with HCC following partial hepatectomy. The Child–Pugh classification was the first systematic and conventional approach used to determine the severity of cirrhosis and select patients who might tolerate hepatic resection. However, it is not always a reliable indicator of hepatic reserve, and has a limited role in predicting postoperative outcome[24]. The MELD score is a reliable measure of mortality risk in patients with end-stage liver disease and is suitable for use as a disease severity index to determine organ allocation priorities. No useful, objective, easily obtained and precise marker has yet been identified to evaluate the general condition of patients before hepatectomy. The ASA grade gives an estimation of organ disease and functional status, and has been suggested as a useful prognostic factor for preoperative patients with HCC[8]. However, it has been criticized for being subjective and imprecise[16].

Sarcopenia is defined as muscle mass two standard deviations below the mean in healthy young adults[25]. Although sarcopenia is associated with ageing, it can also develop as a consequence of chronic disease and malignancy. The European Working Group on Sarcopenia in Older People[15] recommended using the presence of both low muscle mass and low muscle function for the diagnosis of sarcopenia. However, muscle function is difficult to evaluate, and thus low muscle mass was investigated in the present study. There was no correlation between sarcopenia and age, but sarcopenia was significantly correlated with liver dysfunction as indicated by abnormal serum albumin levels and ICGR15 values, as well as with reduced BMI values. There was no correlation between sarcopenia and the Child–Pugh classification, MELD score or liver cirrhosis. There are some reports that serum albumin levels are decreased in patients with sarcopenia[26], which could be an early warning sign of subclinical conditions and impending disease and disability. Montano-Loza and colleagues[12] reported that, of patients with cirrhosis, those with sarcopenia had a significantly lower BMI than patients without sarcopenia. Liver cirrhosis was observed in 50 per cent of patients in their study, in line with the present findings. There is no report concerning the relationship between ICGR15 values and sarcopenia. In one study[12], skeletal muscle area was correlated with MELD score, which would seem to contradict the present findings; however, the mean MELD score was better in the present study, perhaps explaining these findings.

CT is the standard procedure for quantifying skeletal muscle mass, enabling objective and detailed nutritional and metabolic assessment of patients. Moreover, CT is always performed before hepatectomy, allowing precise assessment of sarcopenia. There are some reports that muscle mass as measured by CT is associated with the prognosis of sarcopenia.

It has been suggested previously that surgical outcomes are worse for obese patients[27]; however, there are few reports concerning the effect of being underweight on patient outcomes following hepatectomy for HCC. In this study, lower BMI was correlated with sarcopenia but not with the prognosis. BMI was significantly lower in sarcopenic patients, although only five patients were considered to be underweight (BMI below 18·5 kg/m$^2$). Thus, sarcopenia is not present exclusively in underweight patients.

The molecular mechanism of sarcopenia remains poorly understood. Skeletal muscle was recently identified as an endocrine organ[28]. It has therefore been suggested that cytokines and other peptides are produced, expressed and released by muscle fibres. For example, interleukin (IL) 6 is released from skeletal muscle[28], which may subsequently affect liver metabolism. Both the level and timing of IL-6 release appear to be determining factors for the biological effect in patients with liver fibrosis and HCC[28]. Furthermore, levels of insulin-like growth factor (IGF) 1, which plays a stimulatory role in the development and regulation of skeletal muscle mass[28], are decreased in patients with sarcopenia. In some reports, serum IGF-1 levels were significantly lower in patients with cirrhosis than in healthy subjects, and were correlated with the degree of liver dysfunction. Low serum IGF-1 levels were significantly correlated with advanced clinicopathological parameters, and indicative of poor overall survival in HCC[29]. IGF-1 is produced mainly by the liver, and it may be that serum IGF-1 levels are lower in patients with sarcopenia and that low IGF-1 levels promote the progression of HCC. Further study is needed to clarify the molecular mechanism concerning muscle–liver cross-talk.

It is important to note that, among the significant prognostic factors for overall survival, skeletal muscle mass can be evaluated before hepatectomy. Similarly, skeletal muscle mass, ICGR15, serum DCP level and stage can be evaluated before hepatectomy to prognosticate recurrence-free survival. The identification of patients with sarcopenia before hepatectomy might permit early

preventive strategies to maintain muscle mass, in order to improve prognosis and patient selection for hepatectomy. A recent study indicated that a late evening snack, as an intervention to reduce the fasting phase in patients with cirrhosis, has the potential to improve skeletal muscle proteolysis[30].

## Disclosure

The authors declare no conflict of interest.

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**Supporting information**

Additional supporting information may be found in the online version of this article:

**Table S1** Operative details in patients with hepatocellular carcinoma with, and without sarcopenia (Word document)

**Snapshot quiz**

## Snapshot quiz 13/36

**Answer:** The computed tomography angiogram shows a large right popliteal aneurysm. The options for management are: radiological stenting using a covered stent; and a bypass procedure to exclude the aneurysm. The patient was managed with a bypass procedure from the superficial femoral artery to the below-knee popliteal artery using reversed saphenous vein. The aneurysm was ligated proximally and distally. This aneurysm was deemed unsuitable for radiological stenting owing to the tortuosity of the vessel. The right leg was swollen due to thrombosis of the popliteal vein caused by the pressure effect from the popliteal aneurysm. As this was at least 6 weeks old, the patient did not receive warfarin therapy.

# Third or more repeat hepatectomy for recurrent hepatocellular carcinoma

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***Background.*** *We sought to evaluate the surgical results of third or more repeat hepatectomy for recurrent hepatocellular carcinoma (HCC). The role of repeat hepatectomy for recurrent HCC, especially in cases with third or more repeat hepatectomy, is controversial.*
***Methods.*** *We performed A retrospective, cohort study to analyze the surgical results of repeat hepatectomy performed at a single medical center from 1989 to 2011. A total of 1,000 hepatectomies for HCC were divided into 3 groups: A first hepatectomy group (n = 791), second hepatectomy group (n = 163), and third or more hepatectomy group (n = 46). Operative results and patient prognoses were compared among the 3 groups.*
***Results.*** *There were no differences in early surgical results such as mortality and morbidity among the 3 groups. The 5-year survival rates after the first, second, and third or more hepatectomy were 67%, 60%, and 43%, respectively (P = .1913). There was a significant difference in disease-free survival among the 3 groups, and the 5-year disease-free survival rates after first, second, and third or more hepatectomy were 37%, 29%, and 18%, respectively (P = .0169).*
***Conclusion.*** *Third or more repeat hepatectomy for recurrent HCC was performed safely and associated with relatively long-term survival. Third or more repeat hepatectomy for recurrent HCC seems justified, but high rate of HCC recurrence remains a problem. (Surgery 2013;154:1038-45.)*

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Hepatocellular carcinoma (HCC) is the fifth most common malignancy worldwide, with an annual occurrence of ≥1 million new cases, and is responsible for 500,000 deaths worldwide every year.[1,2] The mainstay of curative treatment for HCC is hepatectomy. With advances in operative techniques and perioperative care,[3,4] the results of hepatectomy for HCC have greatly improved. Nonetheless, the long-term survival after hepatectomy remains unsatisfactory because of the high incidence of intrahepatic recurrence in up to 68–98% of patients.[5] Thus, effective therapeutic strategies for intrahepatic recurrence are critical to prolonging survival after hepatectomy for HCC. In the past 2 decades, repeat hepatectomy has been reported to be safe and to prolong survival after intrahepatic recurrence.[6-14] Recently, salvage liver transplantation was proposed as a curative option for intrahepatic recurrence of HCC, but it is still not widely used because of the insufficient numbers of cadaveric donors and limited availability of appropriate living donors.[15-17] Moreover, the problem of further HCC recurrence after repeat hepatectomy or liver transplantation is adovocated.[17,18] For second recurrence of colorectal liver metastases, third hepatectomy has been reported to be beneficial.[19] However, there have been few reports on further hepatectomy for a second or third recurrence of HCC.[8,10,13,14,20]

Our department has aggressively adopted repeat hepatectomy as the main curative option for treating recurrent HCC, irrespective of the number of recurrences. A retrospective review of patients undergoing hepatectomy for primary and recurrent HCC over 20 years in a single institution was conducted in order to clarify the role of repeat hepatectomy, especially third or more hepatectomy, for recurrent HCC.

## METHODS

**Patients.** A total of 1,000 hepatectomies for HCC were performed at the Department of Surgery, Hiroshima Red Cross and Atomic Bomb

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Survivors Hospital, between January 1989 and March 2010. A flow diagram showing the treatment and outcome of this cohort is provided in Fig 1. Repeat hepatectomy was performed in 209 patients, and consists of a second hepatectomy in 163 patients, third hepatectomy in 36, fourth hepatectomy in 9, and fifth hepatectomy in 1. This series was divided into 3 groups: First hepatectomy ($n$ = 791), second hepatectomy ($n$ = 163), and third or more hepatectomy ($n$ = 46). The medical records of patients in this series were followed until March 2012, and the median follow-up period was 52 months.

**Operative techniques and follow-up methods.** The details of the operative techniques and patient selection criteria for repeat hepatectomy have been reported previously, and are almost identical to those of the initial hepatectomy for primary HCC.[6,21,22] Patients with an indocyanine green dye retention rate at 15 minutes of <40% were selected for hepatic resection, and patients with an indocyanine green dye retention rate at 15 minutes of <35% were selected for anatomic resection.[22] In principle, this selection criteria for hepatic resection was consistent even in the third or more hepatectomy group. Anticoagulant drugs, such as nafamostat mesilate,[23] have been given perioperatively since 1996, and preoperative steroid administration has been routinely performed since 2006.[24]

In almost all hepatic resections, intermittent Pringle's maneuvers consisting of clamping the portal triad for 15 minutes and then releasing the clamp for 5-minute intervals or hemivascular occlusions were applied.[25,26] The clump-crushing method was used to transect the liver parenchyma until 2001, a CUSA system (Valley Lab, Boulder, Colo) has been used since 2002, and a VIO soft-coagulation system (ERBE Elektromedizin, Tübingen, Germany) has been added since 2010.[27] An intraoperative bile leakage test has been routinely performed since 2006.[28] Since 2008, a hyaluronic acid-carboxymethylcellulose membrane (Seprafilm, Genzyme Corporation, Cambridge, MA) was inserted around the liver bed to reduce the adhesion of the duodenum, transverse colon, and omentum to the hepatic hilum.[29]

We examined 5 surgical outcomes among 3 groups: Postoperative mortality, morbidity, duration of hospital stay, overall survival, and disease-free survival. Any death that occurred in the hospital after hepatectomy was recorded as a mortality. Complications were evaluated by Clavien's classification[30] of surgical complications, and those with a score of grade ≥II were defined as positive. After discharge, all patients were examined for HCC recurrence by ultrasonography and tumor markers such as α-fetoprotein and des-γ-carboxy prothrombin every month, and by dynamic computed tomography every 3 or 4 months.[31] No patient received adjuvant chemotherapy or adjuvant lipiodolization in our series. We treated recurrent HCC by repeat hepatectomy,[32] ablation therapy,[33] and lipiodolization[34] according to the previously described strategy.[6]

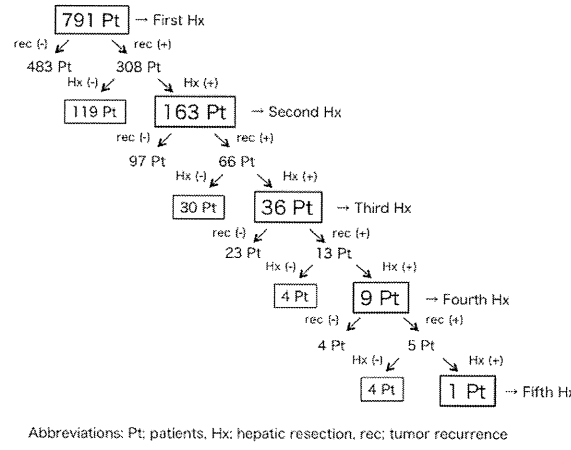


**Fig 1.** Treatment and outcome flow diagram.

**Statistical analysis.** Continuous variables were expressed as mean values ± standard deviation and compared using analysis of variance. Categorical variables were compared using either the Chi-square or Fisher's exact test as appropriate. Survival durations were measured from the last time of operation. Survival curves were generated by the Kaplan–Meier method and compared by the log-rank test. All analyses were performed with Statview 5.0 software (Abacus Concepts, Berkeley, Calif).

## RESULTS

**Comparisons of background characteristics among patients undergoing third or more repeat hepatectomy for recurrent HCC.** The comparisons of patient characteristics among the 3 groups are summarized in Table I. There were significant differences in patient age (first hepatectomy, 65 ± 10 years; second, 68 ± 10 years; third or more, 71 ± 9 years; $P < .0001$). There were no differences in the positive rate of hepatitis B surface antigen (first, 19%; second, 17%; third or more, 20%; $P = .4027$) or hepatitis C virus antibody (first, 64%; second, 69%; third or more, 61%; $P = .6045$). Patients in the second and the third or more hepatectomy groups maintained liver

**Table I.** Comparisons of patients' background characteristics

| *Variables* | *First* (n = *791*) | *Second* (n = *163*) | *Third or more* (n = *46*) | P *value* |
|---|---|---|---|---|
| Age (y) | 65 ± 10 | 68 ± 10 | 71 ± 9 | <.0001 |
| Male/female | 544/247 | 113/50 | 39/7 | .0716 |
| Body mass index | 23.0 ± 3.1 | 22.9 ± 3.1 | 22.3 ± 2.3 | .3118 |
| Diabetes, *n* (%) | 224 (28) | 42 (26) | 13 (28) | .7883 |
| Drinking, *n* (%) | 207 (26) | 38 (23) | 9 (20) | .4027 |
| HBs-Ag+, *n* (%) | 148 (19) | 29 (17) | 9 (20) | .6812 |
| HCV-Ab+, *n* (%) | 508 (64) | 112 (69) | 28 (61) | .6045 |
| Platelets ($\times 10^4/\mu L$) | 18.6 ± 38.4 | 12.8 ± 4.7 | 13.2 ± 4.4 | .1058 |
| Total bilirubin (mg/dL) | 0.8 ± 0.4 | 0.7 ± 0.3 | 0.7 ± 0.3 | .0009 |
| Albumin (g/dL) | 3.9 ± 0.4 | 4.0 ± 0.4 | 4.0 ± 0.4 | .0020 |
| Aspartate aminotransferase (IU/L) | 53 ± 35 | 40 ± 25 | 35 ± 15 | <.0001 |
| Alanine aminotransferase (IU/L) | 53 ± 38 | 40 ± 25 | 35 ± 15 | <.0001 |
| Prothrombin time, *n* (%) | 88 ± 16 | 89 ± 15 | 91 ± 13 | .1649 |
| ICGR-15, *n* (%) | 18.5 ± 10.8 | 19.3 ± 10.5 | 19.7 ± 8.4 | .5119 |
| Child A, *n* (%) | 734 (93) | 156 (96) | 45 (98) | .5089 |

*Ab*, Antibody; *HbS-Ag*, hepatitis B surface antigen; *HCV*, hepatitis C virus; *ICGR-15*, indocyanine green dye retention rate at 15 minutes.

function better and had lower total bilirubin levels than those in the first hepatectomy group (first, 0.8 ± 0.4 mg/dL; second, 0.7 ± 0.3 mg/dL; third or more, 0.7 ± 0.3 mg/dL; $P = .0009$), but there were no differences in Child–Pugh classification (first, A in 93%; second, A in 96%; third or more, A in 98%; $P = .5089$).

**Comparisons of short-term surgical outcomes of third or more repeat hepatectomy for recurrent HCC.** The comparisons of short-term surgical outcomes among the 3 groups are summarized in Table II. The operation time was significantly prolonged in the third or more hepatectomy group (first, 225 ± 98 minutes; second, 232 ± 103 minutes; third or more, 267 ± 86 minutes; $P = .0147$). In the repeat hepatectomy groups, the extent of hepatectomy, such as the resected liver volume (first, 152 ± 214 g; second, 56 ± 61 g; third or more, 47 ± 41 g; $P < .0001$) or positive rate of anatomic resection (first, 41%; second, 21%; third or more, 9%; $P < .0001$) was significantly reduced, and the intraoperative transfusion rate (first, 20%; second, 12%; third or more, 15%; $P = .0376$) was significantly decreased. There were no differences in the hospital mortality rate (first, 1.4%; second, 1.2%; third or more, 0.0%; $P = .7177$), postoperative morbidity rate (first, 31%; second, 26%; third or more, 30%; $P = .4237$), and mean duration of hospital stay (first, 20 ± 16 days; second, 17 ± 20 days; third or more, 16 ± 9 days; $P = .1897$).

**Comparisons of tumor-related factors of third or more repeat hepatectomy for recurrent HCC.** The comparisons of tumor-related factors among the 3 groups are summarized in Table III. There were significant differences in the tumor diameter (first, 3.3 ± 2.4 cm; second, 2.0 ± 0.9 cm; third or more, 1.8 ± 1.0 cm; $P < .0001$) and the number of tumor (first, 1.3 ± 0.8; second, 1.5 ± 1.3; third or more, 1.5 ± 0.9; $P = .0208$). The rate of poorly differentiated HCC was significantly decreased in the repeat hepatectomy groups (first, 30%; second, 17%; third or more, 15%; $P = .0002$), but there were no differences in the positive rate of portal venous infiltration ($P = .0721$) or intrahepatic metastasis ($P = .3162$). The positive rate of histologic cirrhosis also showed no significant differences among the 3 groups (first, 53%; second, 60%; third or more, 52%; $P = .9009$).

**Survival rate after third or more repeat hepatectomies for recurrent HCC.** The overall survival curves after hepatectomy for HCC of the 3 groups are illustrated in Fig 2. There were no differences in overall survival rates, and the 5-year survival rate of the patients who underwent third or more hepatectomy for recurrent HCC reached 43% (first, 67%; second, 60%; $P = .1913$). The disease-free survival curves after hepatectomy for HCC of the 3 groups are illustrated in Fig 3. The disease-free survival rate in the third or more hepatectomy group was significantly decreased, and the 5-year disease-free survival rate of the third or more hepatectomy group remains quite low, that is, 18% (first, 37%; second, 29%; $P = .0169$).

The overall survival and disease-free survival rates after second hepatectomy according to the treatment type (third hepatectomy vs. non-hepatectomy) are illustrated in Fig 4. The overall survival rate is significantly better in the third hepatectomy group, and the 5-year survival rate

**Table II.** Comparisons of surgical outcomes

| *Variables* | *First* (n = *791*) | *Second* (n = *163*) | *Third or more* (n = *46*) | P *value* |
|---|---|---|---|---|
| Surgical outcomes | | | | |
| Operation time (min) | 225 ± 98 | 232 ± 103 | 267 ± 86 | .0147 |
| Blood loss (g) | 681 ± 1062 | 627 ± 805 | 764 ± 797 | .6873 |
| Resected volume (g) | 152 ± 214 | 56 ± 61 | 47 ± 41 | <.0001 |
| Transfusion (%) | 159 (20) | 19 (12) | 7 (15) | .0376 |
| Hr 0:S:1-2 | 468:129:79 | 129:14:4 | 42:2:0 | <.0001 |
| Anatomic resection, *n* (%) | 323 (41) | 34 (21) | 4 (9) | <.0001 |
| sm (mm) | 4.6 ± 6.2 | 3.1 ± 3.9 | 2.4 ± 3.2 | .0013 |
| Postoperative courses | | | | |
| Mortality, *n* (%) | 11 (1.4) | 2 (1.2) | 0 (0.0) | .7177 |
| Morbidity, *n* (%) | 243 (31) | 42 (26) | 14 (30) | .4237 |
| Hospital stay (d) | 20 ± 16 | 17 ± 20 | 16 ± 9 | .1897 |

*Hr 0*, Limited resection; *Hr S*, subsegmentectomy; *Hr 1*, segmentectomy; *Hr 2*, bi-segmentectomy; *sm*, surgical margin.

**Table III.** Comparisons of tumor-related factors

| *Variables* | *First* (n = *791*) | *Second* (n = *163*) | *Third or more* (n = *46*) | P *value* |
|---|---|---|---|---|
| Tumor diameter (cm) | 3.3 ± 2.4 | 2.0 ± 0.9 | 1.8 ± 1.0 | <.0001 |
| Tumor number | 1.3 ± 0.8 | 1.5 ± 1.3 | 1.5 ± 0.9 | .0208 |
| Poorly dif. (%) | 239 (30) | 27 (17) | 7 (15) | .0002 |
| fc+, *n* (%) | 514 (65) | 86 (53) | 21 (46) | .0015 |
| fc-inf+, *n* (%) | 404 (51) | 67 (41) | 20 (43) | .0165 |
| vp, *n* (%) | 436 (55) | 96 (59) | 28 (60) | .0721 |
| im, *n* (%) | 105 (13) | 18 (11) | 3 (7) | .3162 |
| Stage III or IVA, *n* (%) | 93 (12) | 68 (42) | 16 (35) | .0507 |
| α-Fetoprotein (ng/mL) | 14 ± 164 | 28 ± 102 | 18 ± 29 | .6010 |
| Des-γ-carboxy prothrombin (mAU/mL) | 37 ± 57 | 20 ± 50 | 15 ± 52 | .9009 |
| lc+, *n* (%) | 420 (53) | 98 (60) | 24 (52) | .2462 |

*dif*, Differentiation; *fc*, fibrous capsule; *fc-inf*, fibrous capsule infiltration; *vp*, portal venous infiltration; *im*, intrahepatic metastasis; *lc*, liver cirrhosis.

of the non-hepatectomy group remains low (17%). The disease-free survival rate is significantly better in the third hepatectomy group, and the 2-year disease-free survival rate of the non-hepatectomy group remains low (21%). As for the fourth or fifth hepatectomy, 6 of 10 patients (60%) survived >2 years without recurrence, and all patients survived during this follow-up period. However, 11 of 14 patients (79%) with non-hepatectomy treatment for third recurrence or more had early recurrence and 5 of these patients (36%) died within 2 years.

## DISCUSSION

This study comprised a longitudinal observation of surgical results of repeat hepatectomies for recurrent HCC in the largest patient group yet reported, and using a nearly constant strategy over 20 years. Hepatectomy remains as the main option for HCC treatment,[1,2] and repeat hepatectomy for recurrent HCC was first reported to be effective >2 decades ago.[6-14] Hepatectomy remains a complex operative procedure with inherent complications.[3-5] Of course, the more often hepatectomy is repeated, the more difficult the procedures becomes owing to the intra-abdominal adhesions caused by previous hepatectomy. This was also true in our series, in which the mean operation time was significantly prolonged in the third or more hepatectomy group (first, 225 ± 98 minutes; second, 232 ± 103 minutes; and third or more, 267 ± 86 minutes; $P = .0147$). However, we also found there were no differences in early surgical results, such as mortality, morbidity, and the mean duration of hospital stay, among the 3 groups. As for the third or more hepatectomy group, the mortality rate was zero and the mean duration of hospital stay was shortened to 16 days in our series. With meticulous operative procedures and perioperative managements for repeat hepatectomies, third or more hepatectomy for recurrent HCC could be safely performed.[13,20]

Comparing surgical outcomes, the extent of hepatectomy was reduced in the repeat hepatectomy groups. This may have been owing to the smaller tumor diameter in the repeat hepatectomy groups (first, 3.3 ± 2.4 cm; second, 2.0 ± 0.9 cm;

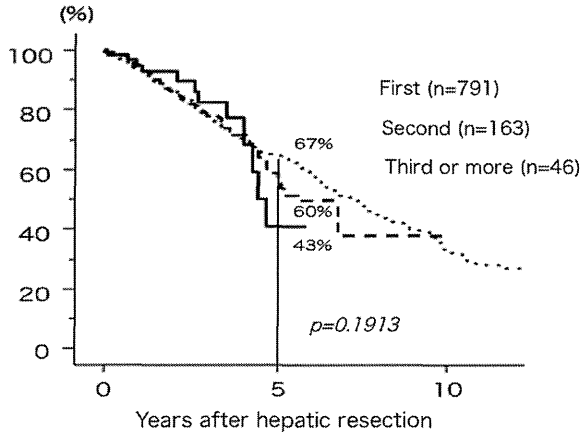


**Fig 2.** Overall survival curves of patients with first hepatectomy for primary hepatocellular carcinoma (HCC), second hepatectomy for recurrent HCC, and third or more hepatectomy for re-recurrent HCC are illustrated. There were no differences between the 3 groups, and the 5-year survival rates were 67% in the first hepatectomy group, 60% in the second hepatectomy group, and 43% in the third or more hepatectomy group is 43% ($P = .1913$).

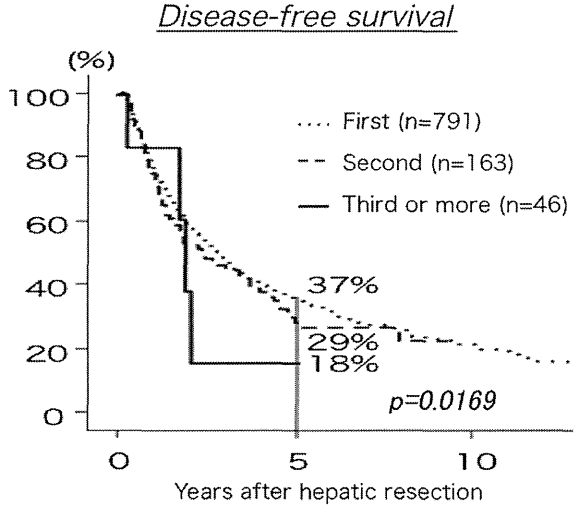


**Fig 3.** Disease-free survival curves of patients with first hepatectomy for primary hepatocellular carcinoma (HCC), second hepatectomy for recurrent HCC, and third or more hepatectomy for re-recurrent HCC are illustrated. Disease-free survival is significantly worse in the third or more hepatectomy group, and the 5-year disease-free survival rates were 37% in the first hepatectomy group, 29% in the second hepatectomy group, and 18% in the third or more hepatectomy group ($P = .0169$).

third or more, 1.8 ± 1.0 cm; $P < .0001$). It is not surprising that the size of HCC removed by repeat hepatectomy was smaller than that removed by first hepatectomy, because most of the tumors were identified during routine follow-up when asymptomatic. Tumor size in HCC is considered to be the most reliable factor for predicting the degree of malignancy,[35] and HCC of ≤2 cm in diameter has low-grade malignancy based on the so-called "stepwise progression" hypothesis.[36] This smaller diameter would help to reduce the surgical stress during third or more hepatectomy for recurrent HCC, and thus was likely among the factors contributing to the good short-term postoperative results. In addition, according to the patient background in Table I, patients with repeat hepatectomies showed a better preservation of liver function. We previously reported that liver dysfunction was a predictive factor linked to postoperative mortality and morbidity.[3,22] The improvements of short-term postoperative results in the repeat hepatectomy groups for recurrent HCC are attributable to the adequate selection of surgical candidates for recurrent HCC.

The overall survival rates of the patients who underwent third or more hepatectomy for recurrent HCC were relatively good, and the 5-year survival rate reached 43% in our series (first, 67%; second, 60%; $P = .1913$). However, the disease-free survival of patients with third or more hepatectomy was significantly shorter than those in the other groups, and the 5-year disease-free survival rate of patients with third or more hepatectomy remains quite low, at 18% (first, 37%; second, 29%; $P = .0169$). Wu et al[20] reported that the more often a repeat hepatectomy for recurrent HCC was performed in an individual patient, the shorter the disease-free interval was thereafter. Irrespective of the high rate of recurrence of HCC in patients with third or more hepatectomy, the overall survival was relatively maintained. Patients with third or more hepatectomy showed a better preservation of liver function (Table I). Patients with good liver function could receive more aggressive and curative treatment for recurrent HCC,[15-17] and this was among the causes of the maintenance of relatively long-term overall survival in patients with third or more hepatectomy for recurrent HCC. On the other hand, there have been several reports in which transfusion was related to the poor prognosis of patients with HCC after hepatectomy.[37-39] The reduced transfusion rate in patients with third or more hepatectomy in our series would also be among the causes for the maintenance of good overall survival in patients with third or more hepatectomy for recurrent HCC.

HCC recurrence is mainly owing to micrometastases or multicentric recurrence.[40,41] In the present series, according to the Kaplan–Meier

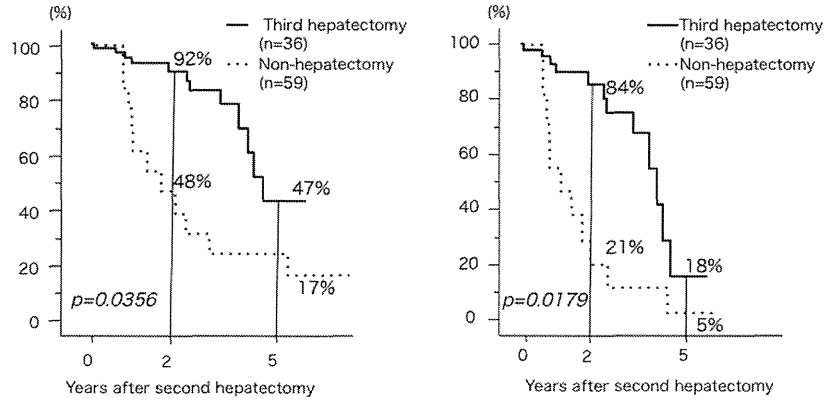


**Fig 4.** Overall and disease-free survivals after second hepatectomy according to treatment type are shown: third hepatectomy versus non-hepatectomy. The overall survival rate is significantly better in the third hepatectomy group, and the 5-year survival rate in the non-hepatectomy group remains low (17%). The disease-free survival rate is significantly better in the third hepatectomy group, and the 2-year disease-free survival rate in the non-hepatectomy group remains low (21%).

curve of disease-free survival in the third or more hepatectomy group, the recurrence after 2 years was drastically increased. Therefore, the remnant liver of patients with third or more hepatectomy for recurrent HCC would have high risk for multicentric recurrence, and preventative measures such as interferon therapy for patients after repeat hepatectomies with chronic hepatitis C would be important.[9] In addition, because of the high rate of recurrence, salvage liver transplantation would be among the treatment choices for recurrence after third or more hepatectomy. However, the drawbacks of liver transplantation include insufficient numbers of cadaveric donors, a lack of appropriate living donors, relatively high mortality of recipients, mortality and morbidity of the living donors, and need for lifelong immunosuppressant therapy and high cost.[15-17,42] In addition, Ng et al[16] reported that the long-term results of nontransplant therapy (5-year survival, 41.8%) and that of liver transplantation (5-year survival, 54%) for transplantable HCC are similar.[16]

With respect to second recurrence, Fig 4 indicates that the overall and disease-free survival rates of patients who underwent third hepatectomy were significantly better than those of patients with non-hepatectomy treatment. But it cannot be concluded definitively that third hepatectomy is superior to other modalities for the treatment of second recurrence of HCC. Comparison of the prognosis after repeat hepatectomy versus other treatments may not be valid because radiofrequency ablation (RFA) or other therapies would be offered to patients on a selection bias. Patients who did not undergo repeat hepatectomy may have had poorer liver functional reserve and/or too advanced recurrent HCC. Chan et al[33] reported that there were no differences in survival between patients with repeat hepatectomy and those with RFA. However, in the same report the 5-year overall and disease-free survival after second hepatectomy for recurrent HCC were fairly low, that is, 35% and 24%, respectively. These data were worse than ours (5-year overall, 60%; 5-year disease-free, 29%). Pathologic examination of totally explanted liver after RFA for HCC showed that complete tumor necrosis rarely occurred (47%) after RFA.[43] To compare the survival impacts of repeat hepatectomy with other treatment modalities such as RFA, a prospective, randomized trial of repeat hepatectomy is needed.

In conclusion, irrespective of the high rate of recurrence, third or more repeat hepatectomy for recurrent HCC was justified, and was performed safely and associated with relatively long-term survival.

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# Preoperative Neutrophil-to-Lymphocyte Ratio Is a Predictor of Survival After Hepatectomy for Hepatocellular Carcinoma

## *A Retrospective Analysis*

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**Objective:** To clarify the prognostic value of the preoperative blood neutrophil-to-lymphocyte ratio (NLR) in patients undergoing hepatectomy for hepatocellular carcinoma (HCC).

**Background:** Although a high NLR has been reported to be a predictor of poor survival in patients with various cancers, it has not been extensively examined in patients with HCC.

**Methods:** This retrospective study enrolled 958 patients who underwent hepatectomy without preoperative therapy for HCC from 1996 to 2009. Clinicopathological parameters, including NLR, were evaluated to identify predictors of overall and recurrence-free survival after hepatectomy. Univariate and multivariate analyses were performed, using the Cox proportional hazards model. The best cutoff was determined with time-dependent receiver operating characteristic curve. To determine the mechanism of NLR elevation, immunohistological examination using CD163 staining was performed in 150 patients.

**Results:** Univariate and multivariate analyses showed that NLR was an independent prognostic factor in overall and recurrence-free survival. The best cutoff of NLR was 2.81, and 238 of 958 patients (24.8%) had NLR of more than 2.81. The 5-year survival rate after hepatectomy was 72.9% in patients with NLR less than 2.81 and 51.5% in those with NLR 2.81 or more ($P < 0.0001$). CD163-positive cell counts were significantly higher in tumors in the group with NLR 2.81 or more than in the group with NLR less than 2.81 ($P = 0.0004$).

**Conclusions:** Our results show that NLR is an independent predictor of survival after hepatectomy in patients with HCC. Accumulation of tumor-associated macrophages in the tumor is associated with a high NLR.

**Keywords:** blood neutrophil-to-lymphocyte ratio, hepatocellular carcinoma, liver resection, prognosis, tumor-associated macrophage

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Hepatocellular carcinoma (HCC) is one of the most common malignant tumors worldwide.[1] Hepatic resection is considered to be the treatment of choice for solitary lesions in patients with noncirrhotic livers or with Child-Pugh–grade cirrhosis, indicating well-preserved liver function.[2] However, the 5-year overall survival rate after hepatic resection is only 50% to 70%.[3–5]

The most significant factor affecting survival is the high postoperative recurrence rate. The reasons for this high recurrence rate remain unclear and seem to be complex and multifactorial.[6,7] One of the important causes of recurrence is metachronous carcinogenesis, caused by hepatic inflammation.[8] Another cause is the malignant potential of cancer cells. Pathological examination shows that microscopic portal vein invasion and intrahepatic metastasis are prognostic factors for survival.[9] Tumor-associated macrophages (TAMs) have been shown to have tumor-promoting effects, with a high density of TAMs in the tumor reported to be associated with a poor prognosis.[10,11] High serum *des-γ*-carboxy prothrombin level and expression of focal adhesion kinase have also been reported to reflect a high malignant potential in HCC.[12,13]

There is increasing evidence that increased systemic inflammation correlates with poorer cancer-specific survival in various cancers.[14–18] Recent studies have shown that the host's inflammatory response to cancer and/or the systemic effects exerted by the cancer cells lead to upregulation of the inflammatory process, predisposing the cancer to proliferation and metastasis through the inhibition of apoptosis, promotion of angiogenesis, and repair of DNA damage.[19,20] The presence of a systemic inflammatory response can be detected by the elevation of the C-reactive protein (CRP) level[21] and neutrophil-to-lymphocyte ratio (NLR)[22]. A high serum CRP level has been shown to be associated with portal vein invasion of cancer cells, and some reports have indicated that a high preoperative serum CRP level is associated with early recurrence of HCC and poorer survival after hepatic resection.[23] A high NLR has been reported to be a predictor of poor survival after hepatic resection, radio-frequency ablation, transarterial chemoembolization, and liver transplantation for HCC.[24–27] To our knowledge, only one relatively small study of fewer than 100 patients by Gomez et al[24] has reported that the preoperative NLR was a prognostic indicator of survival after hepatic resection for HCC.

This study aimed to evaluate the relationship between systemic inflammation and focal infiltration of inflammatory cells, represented by the preoperative NLR and TAMs, and outcome after hepatic resection in 958 patients in 3 high-volume centers in Japan.

## METHODS

### Patients

From January 1996 to December 2009, a total of 422 patients at the Second Department of Surgery, Kyushu University, 253 patients at the Department of Surgery, Hiroshima Red Cross Hospital, and 316 patients at the Department of Surgery, Iizuka Hospital, underwent hepatic resection for HCC. Thirty-three patients who underwent

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preoperative therapy, such as transarterial chemoembolization, radiofrequency ablation, or percutaneous ethanol injection, were excluded, and the remaining 958 patients (689 males, 269 females) were enrolled in this study. The mean age of patients was 67 years.

Curative resection was defined as complete macroscopic removal of the tumor and was performed in 874 patients (91.2%). Of these, 591 patients (61.7%) were seropositive for hepatitis C antibody (HCV-Ab), 161 (16.8%) were seropositive for hepatitis B surface antigen (HBs-Ag), 204 (21.3%) were seronegative for both HCV-Ab and HBs-Ag, and 5 (0.5%) were seropositive for both HBs-Ag and HCV-Ab. Of the 422 patients who underwent hepatic resection in Kyushu University, 150 consecutive patients who underwent resection from January 1997 to March 2005 were selected for immunohistological examination using CD163 staining.

### Prognostic Factors in Overall and Recurrence-Free Survival After Hepatectomy

Neutrophil-to-lymphocyte ratios of all the patients in this study were calculated on the basis of preoperative blood value. Univariate analysis in overall survival and recurrence-free survival was performed, using the Cox proportional hazards model. The overall survival was evaluated in all the 958 patients, and the recurrence-free survival was evaluated only in 874 patients who underwent curative resection. The following variables were examined with respect to overall survival and recurrence-free survival rate: age, sex, serum albumin level, indocyanine green retention rate at 15 minutes (ICGR15), tumor size, serum $\alpha$-fetoprotein (AFP) level, portal vein thrombus, number of tumors, TNM stage according to the Liver Cancer Study Group in Japan[28] (I or II vs III or IV), and curative resection (resection without remnant tumors). In the analysis of recurrence-free survival, variable: curative resection was excluded, because postoperative recurrence was defined only in the patients without remnant HCC who underwent curative resection. The contiguous variables were entered into the model.

The best cutoff of NLR was determined by receiver operating characteristic curve. The recurrence pattern of HCC was compared between patients with the best cutoff value of NLR. The recurrence pattern was defined as nodular ($\leq$3 nodules), multiple (>3 nodules), and extrahepatic metastasis (metastasis to organs other than the liver), as previously described.[29]

### Follow-up Strategy and Recurrence Pattern

After discharge, all patients underwent monthly screening for recurrence, using ultrasonography and tumor markers such as AFP, and 6-monthly computerized tomography scanning. If recurrence was suspected, additional investigations such as hepatic angiography were performed.

### Immunohistochemical Examination

Sections of resected specimens were fixed in 10% buffered formalin, embedded in paraffin, and stained using the Envision+ system and DAB kit (DAKO, Grostrup, Denmark). Immunohistochemical staining was performed using CD163 antibodies (10D6, 1:200; Novocastra). Sections were pretreated before being incubated with primary antibodies in a microwave oven for 20 minutes. Serial sections were stained and examined by 2 pathologists (Y.M. and S.A.). The total number of cells with cytoplasmic or membrane staining in 3 high-power fields was counted.

### Statistical Analysis

All data are expressed as the mean $\pm$ standard deviation. Independent $\chi^2$ tests were used to compare categorical variables. Continuous variables were compared using unpaired $t$ tests. Survival curves were analyzed using the Kaplan-Meier method and compared using the log-rank test. The Cox proportional hazards model was used for univariate and multivariate analyses. The best cutoff of NLR was determined by time-dependent receiver operating characteristic curve.[30] Adjustment for covariates and the Cox proportional hazards model was conducted using JMP software (SAS Institute, Cary, NC) on a Windows computer. $P$ values of less than 0.05 were considered statistically significant.

## RESULTS

### NLR as an Independent Prognostic Factor

The statistically significant prognostic factors identified by univariate analyses are shown in Table 1. Indicators of poor liver function, such as low serum albumin level and high ICGR15, were identified as significant predictors of poor prognosis. Among tumor-related factors, large tumor size, high AFP level, presence of portal vein thrombus, multiple tumors, advanced clinical stage, and noncurative resection were identified as predictors of poor prognosis. Furthermore, NLR was also identified as a predictor of prognosis. Multivariate analyses identified low serum albumin level, large tumor size, high NLR level, presence of portal vein thrombus, multiple tumors, and advanced clinical stage as independent predictors of poor prognosis (Table 2).

The statistically significant factors in recurrence-free survival identified by univariate analyses are shown in Table 3. Indicators of poor liver function, such as low serum albumin level and high ICGR15, were identified as significant predictors of poor prognosis. Among tumor-related factors, large tumor size, high AFP level,

**TABLE 1.** Univariate Analyses of Factors in Relation to Overall Survival, Using the Cox Proportional Hazards Model

| Prognostic Variables | Hazard Ratio | P | 95% CI |
|---|---|---|---|
| Age | 1.226 | 0.4145 | 0.993–1.018 |
| Sex | 1.964 | 0.6105 | 0.821–1.400 |
| Albumin | 7.813 | <0.0001 | 0.271–0.457 |
| ICGR15, % | 3.274 | 0.0011 | 1.007–1.030 |
| Tumor size | 8.527 | <0.0001 | 1.117–1.193 |
| AFP | 5.608 | <0.0001 | 1.000–1.000 |
| Portal vein thrombus | 7.666 | <0.0001 | 0.194–0.378 |
| Multiple | 5.520 | <0.0001 | 0.375–0.627 |
| Stage (I + II) | 8.150 | <0.0001 | 0.292–0.471 |
| NLR | 3.716 | 0.0002 | 1.022–1.074 |
| Curative resection | 2.392 | 0.0168 | 0.445–0.923 |

**TABLE 2.** Multivariate Analyses of Factors in Relation to Overall Survival, Using the Cox Proportional Hazards Model

| Prognostic Variables | Hazard Ratio | P | 95% CI |
|---|---|---|---|
| Albumin | 6.779 | <0.0001 | 0.279–0.495 |
| NLR | 3.745 | 0.0002 | 1.027–1.088 |
| Tumor size | 3.736 | 0.0002 | 1.036–1.122 |
| Portal vein thrombus | 3.445 | 0.0006 | 0.315–0.728 |
| Stage (I + II) | 2.603 | 0.0092 | 0.467–0.898 |
| Multiple | 2.211 | 0.0270 | 0.512–0.960 |
| ICGR15 | 1.532 | 0.1254 | 0.997–1.022 |
| Curative resection | 1.044 | 0.2967 | 0.534–1.211 |
| AFP | 1.000 | 0.5100 | 1.000–1.000 |
| Age | 1.003 | 0.6721 | 0.990–1.016 |
| Sex | 1.058 | 0.6947 | 0.797–1.405 |

presence of portal vein thrombus, multiple tumors, and advanced clinical stage were identified as predictors of poor prognosis for recurrence-free survivals. Furthermore, NLR was also identified as a predictor of tumor recurrence. Multivariate analyses identified high AFP levels, low serum albumin level, high IGGR15, high NLR level, and presence of portal vein thrombus as independent predictors of tumor recurrence (Table 4).

## Selection of the Best Cutoff Point for NLR

The best cutoff of NLR was determined for postoperative prognosis, using time-dependent receiver operating characteristic curve. An NLR of 2.81 was the best cutoff point for operative prognosis. All the patients were divided into 2 groups: a low (<2.81) NLR group (n = 720) and a high (≥2.81) NLR group (n = 238).

## Prognostic Comparisons of the Low and High NLR Groups

The overall survival rates of patients in the low and high NLR groups are shown in Figure 1. The overall 1-, 3-, and 5-year survival rates were 95.5%, 83.9%, and 72.9% in the low (<2.81) NLR group and 87.1%, 68.9%, and 51.5% in the high (≥2.81) NLR group, which was a significant difference ($P < 0.0001$). The mean survival time was 8.0 ± 0.23 years in the low NLR group and 6.1 ± 0.38 years in the high NLR group.

The recurrence-free survival rates of patients in the low and high NLR groups are shown in Figure 2. The recurrence-free survival rate was significantly higher in the low NLR group than in the high NLR group ($P = 0.0272$).

Comparison of tumor recurrence patterns between the groups is shown in Table 5. Considering those patients with recurrence,

**TABLE 3.** Univariate Analyses of Factors in Relation to Recurrence Free Survival, Using the Cox Proportional Hazards Model

| Prognostic Variables | Hazard Ratio | *P* | 95% CI |
|---|---|---|---|
| Age | 1.002 | 0.6467 | 0.993–1.011 |
| Sex | 1.121 | 0.2622 | 0.919–1.366 |
| Albumin | 3.928 | <0.0001 | 0.546–0.817 |
| ICGR15, % | 3.603 | 0.0003 | 1.007–1.024 |
| Tumor size | 1.452 | 0.1465 | 0.991–1.063 |
| AFP | 6.271 | <0.0001 | 1.000–1.000 |
| Portal vein thrombus | 2.659 | 0.0078 | 0.452–0.887 |
| Multiple | 2.657 | 0.0079 | 0.580–0.921 |
| Stage (I + II) | 3.438 | 0.0006 | 0.561–0.854 |
| NLR | 2.359 | 0.0183 | 1.005–1.059 |

**TABLE 4.** Multivariate Analyses of Factors in Relation to Recurrence free Survival, Using the Cox Proportional Hazards Model

| Prognostic Variables | Hazard Ratio | *P* | 95% CI |
|---|---|---|---|
| AFP | 5.376 | <0.0001 | 1.000–1.000 |
| Albumin | 3.517 | 0.0004 | 0.551–0.844 |
| ICGR15 | 2.509 | 0.0121 | 1.003–1.021 |
| NLR | 2.096 | 0.0361 | 1.002–1.060 |
| Portal vein thrombus | 2.337 | 0.0194 | 0.487–1.032 |
| Multiple | 2.211 | 0.0728 | 0.512–0.960 |
| Stage (I + II) | 2.603 | 0.2673 | 0.659–1.123 |
| Sex | 1.096 | 0.368 | 0.892–1.345 |
| Tumor size | 1.008 | 0.8641 | 0.965–1.044 |
| Age | 1.003 | 0.9082 | 0.991–1.010 |

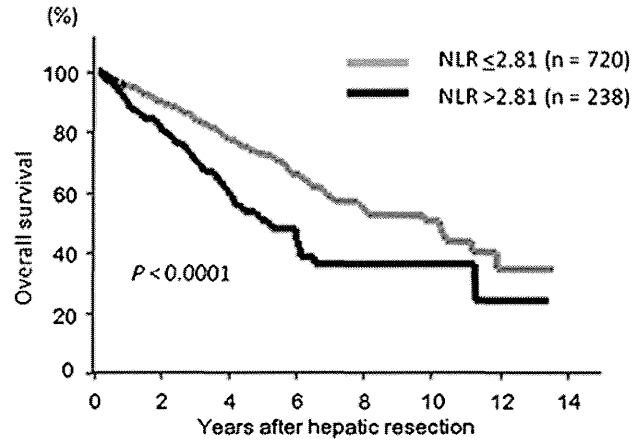


**FIGURE 1.** Comparison of overall survival rates in the low (<2.81) and high (≥2.81) blood NLR groups. The overall 1-, 3-, and 5-year survival rates were 95.5%, 83.9%, and 72.9% in the low (< 2.81) NLR group and 87.1%, 68.9%, and 51.5% in the high (≥2.81) NLR group, which was a significant difference ($P < 0.0001$).

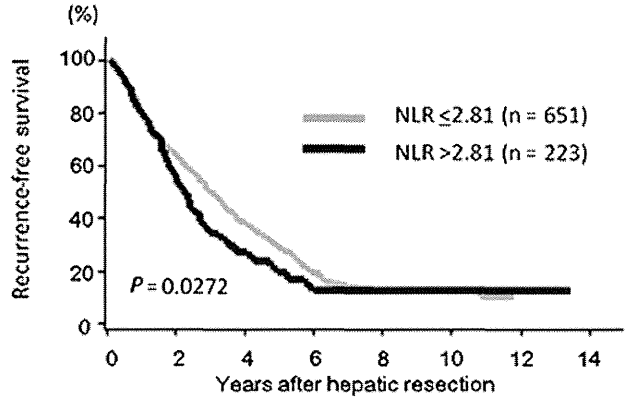


**FIGURE 2.** Comparison of recurrence-free survival rates in the low (<2.81) and high (≥2.81) NLR groups. The recurrence-free survival rate was significantly higher in the low NLR group than in the high NLR group ($P = 0.0272$).

**TABLE 5.** Comparison of Recurrence Patterns Between the Low and High NLR Groups

| NLR | Nodular | Multiple | Extrahepatic | *P* |
|---|---|---|---|---|
| <2.81 (n = 351) | 243 (69.2%) | 86 (24.5%) | 22 (6.3%) | 0.0002 |
| ≥2.81 (n = 115) | 55 (47.8%) | 48 (41.7%) | 12 (10.4%) | — |

Nodular indicates fewer than 3 recurrent intrahepatic tumors; multiple, 3 or more recurrent intrahepatic tumors.

multiple tumors in the liver were significantly more frequent in the high NLR group than in the low NLR group ($P = 0.0002$).

## Immunohistochemical Examination

We performed immunohistochemical staining for CD163 in 150 consecutive cases at Kyushu University Hospital. Figure 3A

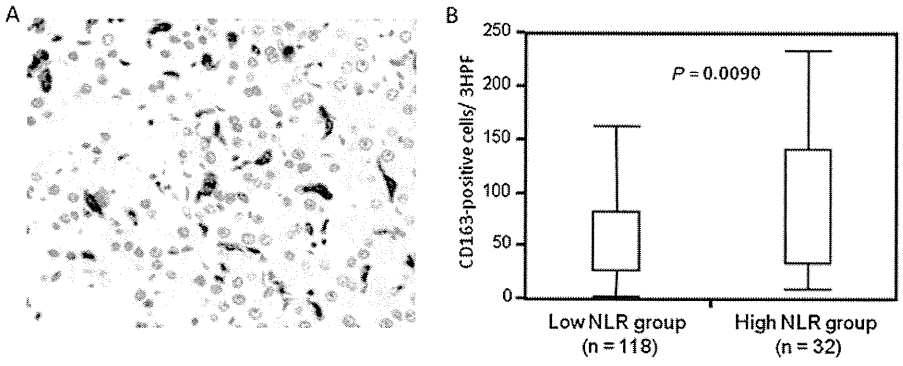


**FIGURE 3.** A, Immunohistochemical CD163 staining of a hepatocellular carcinoma specimen (×200). B, CD163-positive cell counts in the low and high NLR groups ($P = 0.0090$). HPF indicates high-power field.

shows CD163 staining of TAMs. We compared tumor infiltration by CD163-positive cells between the high and low NLR groups. CD163-positive cell counts were significantly higher in tumors in the high NLR group than in the low NLR group (91.0 ± 82.5 vs 61.2 ± 47.4, $P = 0.0090$; Fig. 3B).

## DISCUSSION

Indicators of poor liver function, such as low serum albumin level and high ICGR15, and tumor invasion factors, such as large tumor size, presence of portal vein thrombosis, multiple HCC, and high serum AFP level, have previously been reported to be predictors of poor prognosis in patients with HCC.[31,32] The results of this study clearly show that the high preoperative NLR was an independent predictor of poor survival after hepatectomy in patients with HCC.

Although a high NLR is thought be associated with systemic inflammation, the cause of this inflammation remains unclear. Hashimoto et al[22] reported that a high CRP level was an independent prognostic factor in patients who underwent hepatectomy for HCC. Fever and high CRP level are suspected to be caused by humoral factors, especially inflammatory cytokines such as interleukin (IL)-6, IL-8, and tumor necrosis factor-$\alpha$. However, fever is extremely rare in patients with HCC, and this mechanism cannot be applied to all patients with a high NLR.

Some reports have indicated that macrophage infiltration into HCC is related to the aggressiveness of the tumor.[10,11] Macrophages can assume a range of different phenotypes based on environmental stimuli. The extremes of this range in vitro are the M1 phenotype, associated with active microbial killing, and the M2 phenotype, associated with tissue remodeling and angiogenesis.[10,11,32] When monocytes in the tumor are exposed to tumor-derived anti-inflammatory molecules such as IL-4, IL-10, transforming growth factor-$\beta$1, and prostaglandin E2, they polarize into M2 macrophages.[11] The M2 phenotype macrophage seems to be the dominant type in tumors, with TAMs characterized by high expression of M2 macrophage antigens such as CD163 and high constitutive expression of IL-6 and IL-10.[33,34] Our immunohistochemical analysis showed that high infiltration of TAMs was associated with a high NLR. TAMs express some cytokines, such as IL-6 and IL-8, within the lesion, and these cytokines may promote systemic neutrophilia.[35–37] Ubukata et al[38] demonstrated that a high NLR is significantly correlated with high numbers of Th2 cells in patients with gastric cancer. Th2 cells express IL-4 and IL-10, which polarize macrophages to TAMs. A high NLR is associated with a high infiltration of TAMs and high inflammatory cytokine production in the tumor. On the contrary, our histological examination revealed that local accumulation of neutrophils into HCC might not play an important role in NLR elevation (date not shown). This phenomenon may be explained by complex expression of several cytokines. Kuang et al[39] demonstrated that intratumoral neutrophils did not have a critical role in tumor progression but peritumoral neutrophils did, and proinfilammatory IL-17 secreted by lymphocytes recruits neutrophils to peritumoral stroma. IL-17 is one of the proinflammatory cytokines. Peritumoral IL-17 may enhance systematic neutrophils in our study. Close relationship between TAMs and IL-17–producing cells was reported previously.[34,40] Thus, similar mechanism may be one of the cause of NLR elevation in HCC patients. From this point of view, a high infiltration of TAM is a first and important step of NLR elevation. Further examination is necessary to determine this clear mechanism.

There are a many reports regarding the promotion of distant metastasis of cancer cells by TAMs. Rolny et al[41] demonstrated that inhibition of TAM infiltration into tumors, by neutralizing antibodies to monocyte chemoattractants, reduces metastasis. Recent studies have provided evidence that TAMs and cytokines, such as IL-1, tumor necrosis factor, IL-6, and IL-8, increase metastasis. IL-6 levels are much higher in HCC patients than in healthy adults.[42] Harimoto et al[43] reported an HCC patient with a high IL-8 level, high CRP level, and pyrexia who had an extremely poor outcome after hepatectomy. Liu et al[44] demonstrated that IL-6 induced antiapoptotic activity via the STAT3 signaling pathway in human HCC cell lines. These phenomena may be related to TAMs, which can produce IL-6 and IL-8. Anti-inflammatory treatment may be beneficial in the treatment of HCC, and further study is necessary to investigate this.

## CONCLUSIONS

Neutrophil-to-lymphocyte ratio is an easily measurable inflammatory biomarker. Our results show that NLR is an independent predictor of survival after hepatectomy in patients with HCC and that accumulation of TAMs in the tumor may be one of the causes of NLR elevation.

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LETTER FROM THE FRONTLINE

# Fairly Rare Spontaneous Disappearance of a Hepatic Artery Aneurysm Following Living Donor Liver Transplantation

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## TO THE EDITORS

The patient was a 54-year-old female with end-stage liver disease secondary to primary biliary cirrhosis without a hepatic artery aneurysm. She underwent ABO-incompatible living donor liver transplantation (LT) with a left lobe graft donated by her 58-year-old husband. Preoperatively, the patient underwent plasma exchange several times and rituximab administration for the removal of anti–blood type antibodies. The hepatic artery of the graft (A2/3) was anastomosed end to end to the recipient's left hepatic artery, and A4 was anastomosed to the middle hepatic artery. The cold and warm ischemia times were 67 and 39 minutes, respectively. Postoperative immunosuppression was induced with cyclosporine with mycophenolate mofetil and steroids. Routine follow-up dynamic computed tomography 1 week after LT revealed no hepatic artery aneurysm (Fig. 1A). However, a tiny globular pseudoaneurysm at the distal side of the anastomosis with the thrombus at the main trunk of the portal vein was revealed 2 weeks after LT (Fig. 1B). Coumadin administration at 2 mg/day was initiated, and good control was achieved with an international normalized ratio of 1.5 to 2.0; this prevented the development of the portal thrombus. The pseudoaneurysm developed with a spindle-shaped form 1 month after LT (Fig. 1C), and 2 months after LT, it had a diameter of 7 mm (Fig. 1D). Open surgery for resecting and reconstructing the pseudoaneurysm was planned. However, a computed tomography examination revealed the spontaneous disappearance of the hepatic artery pseudoaneurysm 10 days after a pause in the anticoagulant administration (Fig. 1E). There was no new development of the pseudoaneurysm 1 month after its disappearance.

## DISCUSSION

A hepatic artery pseudoaneurysm is an unusual and potentially serious complication that can occur after LT, and it is characterized by a high mortality rate.[1] Early diagnosis and treatment (eg, surgical reconstruction and catheter-based endovascular treatment of stent or coil embolization) are essential for preventing life-threatening hemorrhaging.[2] However, these therapies involve considerable associated risks.[3] The mechanism of hepatic artery pseudoaneurysm development after LT is usually a technical problem involving a bacterial infection and inflammation around the hepatic artery, which cause weakening of the vessel wall.[1-3] In the case reported here, there was excessive local anticoagulant around the hepatic artery anastomosis site, which may have been unable to adapt to any qualitative or quantitative changes because of decreased elasticity and strength. The minute intimal hemorrhage consequently may have induced the development of the hepatic artery pseudoaneurysm.[3,4] In this case, the sequence of anticoagulant treatment, treatment of the portal thrombus, and no surgical resection of the pseudoaneurysm allowed the development of the hepatic pseudoaneurysm and its later disappearance to be observed for the first time. The pseudoaneurysm developed first as a tiny, spindle-shaped form before it became a larger globular body and vanished without a trace. If the anticoagulation had been discontinued earlier as the pseudoaneurysm was developing from the spindle-shaped form, the risk of rupture would have been very low.

Fistouris et al.[4] extensively reviewed their cases and showed that an infectious etiology (particularly bile leakage) may be closely related to the occurrence of pseudoaneurysms.[4] They also showed the major

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The authors declare that they have no conflicts of interest.

The study protocol conformed to the ethical guidelines of the 1975 Helsinki Declaration and was approved by our institutional review board.

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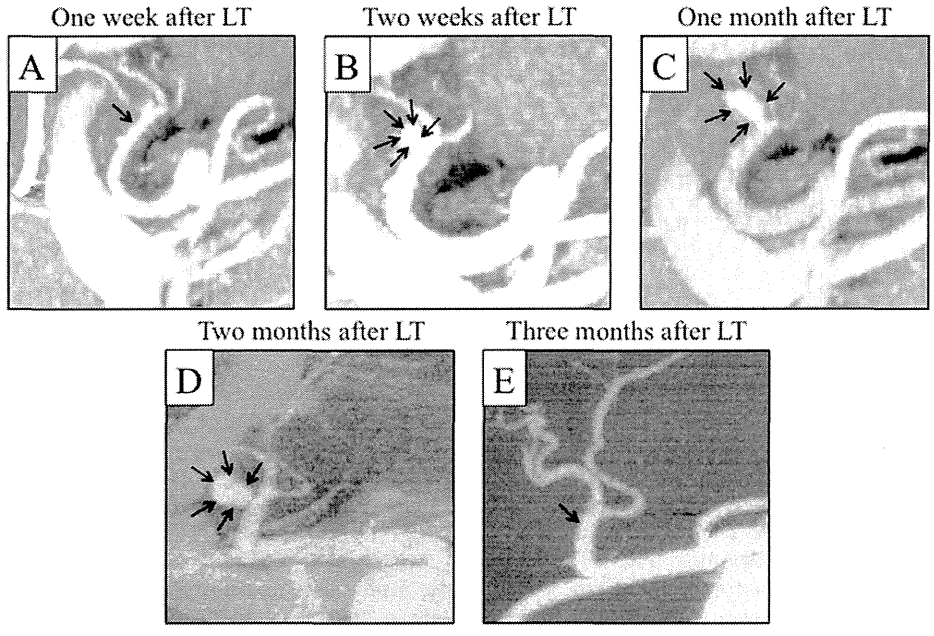


Figure. 1. Development of a hepatic artery pseudoaneurysm as observed with dynamic computed tomography after LT. (A) One week after LT, there was no hepatic artery aneurysm. (B) Two weeks after LT, a tiny globular pseudoaneurysm was evident at the distal side of the anastomosis. (C) One month after LT, the pseudoaneurysm was developing as a spindle-shaped form. (D) Two months after LT, the pseudoaneurysm had grown to 7 mm in diameter. (E) Three months after LT, the hepatic artery pseudoaneurysm spontaneously disappeared (10 days after a pause in the anticoagulant administration).

responsible bacterium to be *Candida albicans* and identified hepaticojejunostomy as one of the risk factors. Molecular biological analysis has shown that tumor necrosis factor α production from endothelial cells, which are often highly expressed in infectious insults, may prevent the fibrotic organization of the internal elastic lamina and aggravate hepatic artery pseudoaneurysms.[5] In light of such evidence, only the manipulation of the anticoagulant series could have clinically caused the pseudoaneurysm in this case because there were no intraoperative and postoperative infectious insults.

Here we report a rare case of a hepatic artery pseudoaneurysm that disappeared after living donor LT. This case suggests that a wait-and-see strategy may be appropriate with careful case-by-case consideration when an anticoagulant treatment is being used.

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