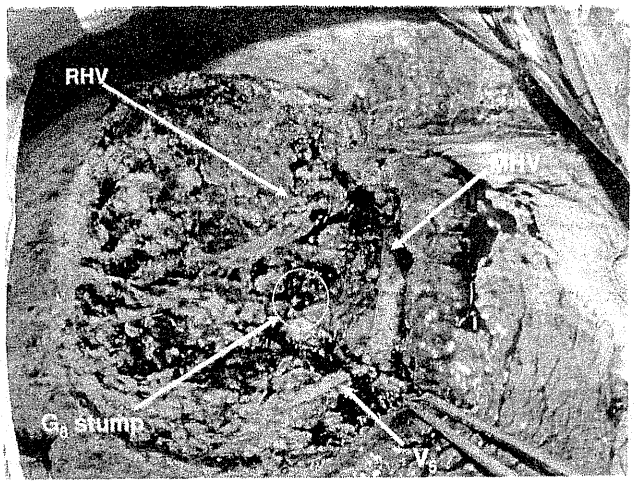


**Fig. 4** Standard view after the anatomical resection of segment 8. The landmark veins were longitudinally exposed in the cutting surface; the right hepatic vein (RHV), the middle hepatic vein (MHV), and drainage vein of segment 5 (V5). The stumps of the branch of Glisson's triad in segment 8 are seen

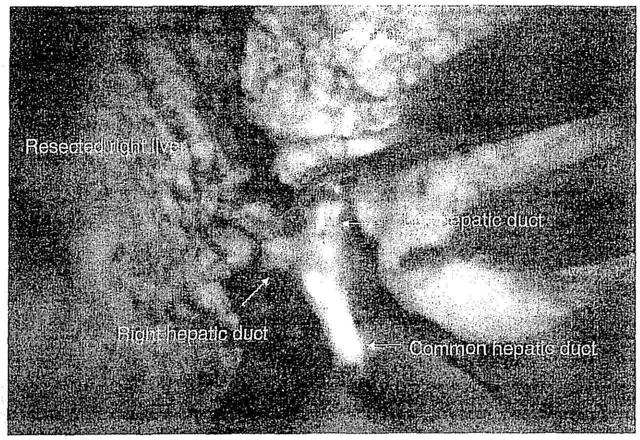


**Fig. 6** The PDE view during right hepatectomy 2 h after venous ICG injection. ICG excretion into the bile from the parenchyma was monitored by PDE; the cutting delineation for the right hepatic duct was clearly visible

## Discussion

Several studies on hepatocellular carcinoma (HCC) have demonstrated a survival benefit for anatomical liver resection [10, 11] because it usually arises within one segment of the liver and the entire functional liver unit can be easily removed. The development of segment-based resection using intrahepatic glissonian access enabled the development of techniques that identify and isolate the right and left segmental glissonian pedicles. These techniques permit a tailored liver resection by removing only the involved liver segments. This technique for performing an anatomic resection of the liver segments using the CEUS and ICG-fluorescent navigation system has been described in a previous report [10]. Careful avoidance of the anatomical landmarks of the liver segments during resection prevents impaired vascularization of the remaining parenchyma and excessive bleeding [10, 11]. The anatomic resection technique, based on specific anatomical landmarks following a control of a Glissonian pedicle with hilar dissection, is important, and fluorescence navigation with ICG and contrast-enhanced intra-operative ultrasound with Sonazoid is thus considered to be a useful tool for safe and systematic hepatic resection.

ICG is a fluorescent dye administered by intravenous injection for optical arteriography and the visualization of superficial vessels [4]. Recently, experimental and preliminary clinical studies have suggested that ICG can

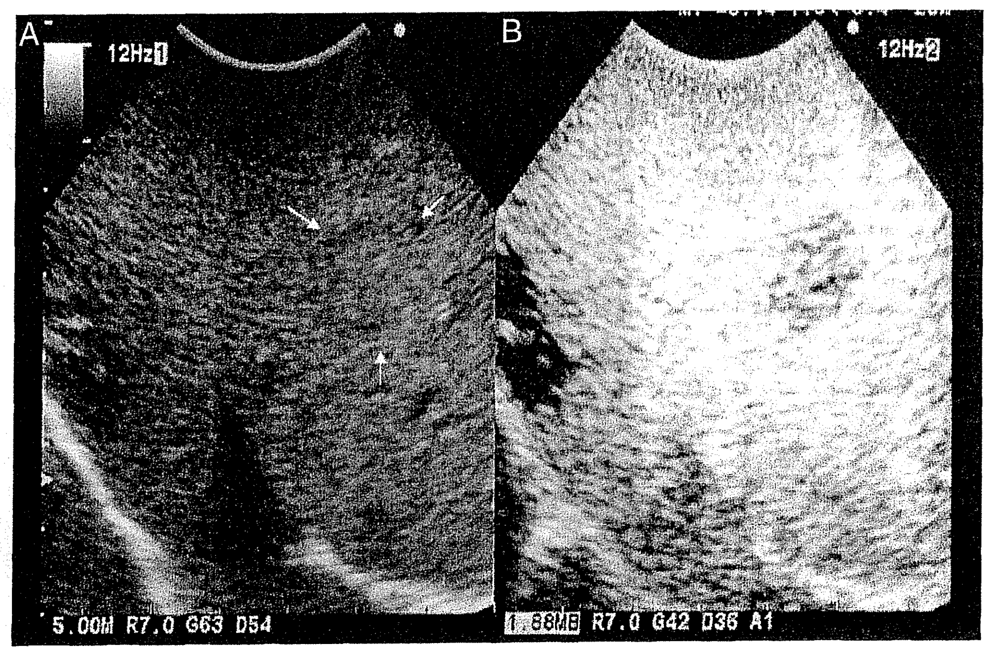


**Fig. 5** IOUS and CE-IOUS images of HCC metastasis with liver cirrhosis at segment 7. **a** A metastatic lesion was detected as a slightly hypoechoic mass which could not be differentiated from the surrounding fibroid indurations based on the liver cirrhosis. **b** The CE-IOUS view of the same lesion shows a clear hypoechoic mass at the late Kupffer phase

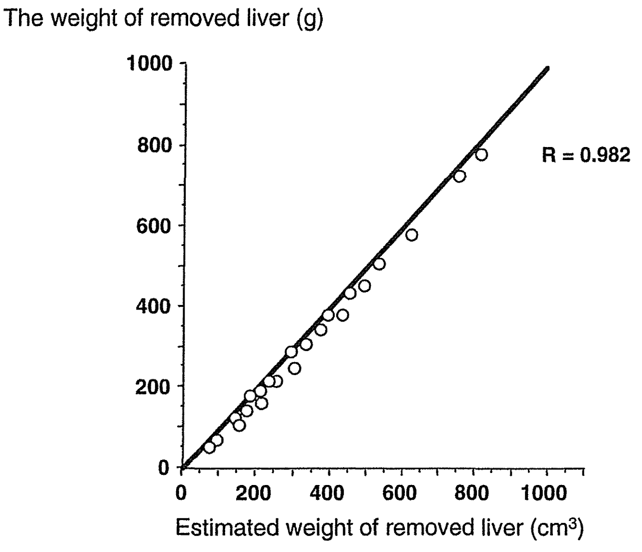


**Fig. 7** The correlation diagram between the weight of the removed specimen and the estimated volume of the resected specimens based on preoperative 3D-CT using this software program

be used for near-infrared (NR) fluorescence imaging for identifying liver metastasis, especially lesions located on the liver surface [12]. Using a PDE system with an ICG flow, the authors transcutaneously observed the liver in real time. In our study, after ligation of the portal pedicle, the demarcation line could not be easily detected as a slightly dark line in five patients (23%), which prevented its differentiated from the non-ischemic area due to liver cirrhosis with type B or C hepatitis. The reason about showing the visible unclear demarcation line in cirrhotic patients may be owing to the decrease of whole blood flow in liver with fibrosis. However, when viewed with the PDE system, the same lesion revealed the ischemic area to be a negative-brightness area. There were no complications resulting from the PDE and CE-IOUS procedure, even in the patients with cirrhosis. The present study confirmed that intra-vascular injection of ICG following a control Glissonian pedicle can be used to identify landmarks of the liver surface in patients for anatomical liver resection, and that this fluorescence ICG navigation system is a safe and highly sensitive method for assessing the regions of liver demarcation. ICG is cleared rapidly from the liver after venous injection, with an extremely short half-life in the blood (<3.4 min) [13]. The high levels of liver uptake require almost 2 h before ICG fluorescence in bile begins to exceed that in the liver, and results in a statistical correlation between NR fluorescence in the liver and subsequent ICG excretion into the bile [14]. ICG is a better choice for bile duct imaging because there is adequate time for clearance (i.e., at least 2 h) during the resection of liver parenchyma for detecting the cutting portion of bile duct. The NR fluorescent system for imaging both the liver parenchyma and the extrahepatic biliary duct can thus be applied during surgery to permit real-time, image-guided assessment of the anatomy and function of the extrahepatic bile ducts. Similar results from the fluorescent imaging of liver segments [15] and the extrahepatic bile ducts utilizing biliary excretion of ICG [16] were recently reported. Aoki et al. [13] previously reported that the fluorescence imaging technique for identification of segments and subsegments of the liver described herein has the advantages of safety and reproducibility, and that this technique provided insights into the imaging system for either subsegmentectomy or segmentectomy in patients with malignant hepatic neoplasms. However, the potential difference from the current paper is the point of combining use of PDE and CE-IOUS. With regard to the benefit of using both methods after clamping of the pedicle, clear discoloration marks on the liver surface could be confirmed with the PDE system, and the resectional line of the parenchyma could be confirmed with CE-IOUS at any time during hepatectomy.

Intra-operative ultrasonography (IOUS) is performed in a systematic fashion, particularly for inspecting the liver parenchyma for previously diagnosed and new nodules, identifying any major vascular or biliary involvement and to establish the surgical strategy. Secondly, contrast-enhanced IOUS (CE-IOUS) is carried out for lesion characterization and for the detection of new nodules. To find the sectional or segmental margin in a parenchyma during hepatectomy, CE-IOUS was performed using the new microbubble agent Sonazoid™, which provides a parenchyma-specific contrast image based on its accumulation in Kupffer cells, and is stable enough to allow image acquisition under ultrasound exposure for more than 30 min. Following the portal pedicle ligation via a posterior intrahepatic approach for anatomical hepatectomy, the normal liver parenchymal cells were uniformly enhanced except for the segment with the loss of blood flow, as detected by CE-IOUS. Furthermore, the segment to be removed was identified to be a filling defect during a hepatectomy. CE-IOUS with Sonazoid™ can be applied to anatomical hepatectomy to permit real-time, image-guided assessment of the patient's anatomical features.

The present results suggested that the concomitant use of CE-IOUS with the Sonazoid™ and PDE system may be a safe and more sensitive method that can be used to visualize the liver in addition to the performance of preoperative CT or MRI. In conclusion, the present study may demonstrate the clinical value of the combined use of a fluorescence ICG navigation system and contrast-enhanced intraoperative ultrasonography image using Sonazoid™, which is a novel modality for the detection of liver sections and segments during an anatomical hepatic resection.

**Conflicts of interest** None.

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# Triple Positive Tumor Markers for Hepatocellular Carcinoma Are Useful Predictors of Poor Survival

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**Objective:** To determine the importance of the expression pattern of multiple tumor markers for hepatocellular carcinoma (HCC) with regard to the tumor malignancy and patient survival.

**Background:** Several studies have indicated that HCC tumor markers, including alpha-fetoprotein (AFP), *Lens culinaris* agglutinin-reactive fraction of AFP and des-$\gamma$-carboxy prothrombin were predictors of HCC malignancy. However, few reports have shown the relevance of the expression pattern of these 3 tumor markers with regard to patient prognosis. We herein reported the influence of the expression pattern of these 3 tumor markers on HCC malignancy and patient prognosis.

**Methods:** This retrospective study analyzed 185 patients who underwent hepatectomy for HCC between January 1999 and May 2009. The relationships between clinical parameters and these 3 tumor markers were analyzed. Cox proportional hazards regression analyses were performed to estimate risk factors for recurrence and survival. Furthermore, the relationships between pathological parameters and the expression patterns of the 3 tumor markers were analyzed.

**Results:** From clinical parameters, expression patterns of 3 tumor markers were related to maximum tumor size and macrovascular invasion in image findings. Multivariate analyses revealed independent risk factors for recurrence or survival to be the Child-Pugh score, the presence of multiple tumors, and triple positive tumor marker expression. From pathological findings, microvascular invasion and an Edmondson-Steiner classification of III or IV were related to the expression patterns of the 3 tumor markers.

**Conclusions:** Triple positive tumor markers for HCC showed poor prognosis and invasive characteristics in pathological findings. Examination of these markers would be useful for predicting the degree of HCC malignancy.

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Hepatocellular carcinoma (HCC) is the sixth most common malignancy worldwide, and it is especially common in southern and eastern Asia. It is also the third most common cause of death from cancer.[1,2] In general, the most potent diagnostic methods for HCC are imaging studies, such as ultrasonography, computed tomography (CT), magnetic resonance imaging (MRI), and angiography. Tumor staging is conducted predominantly based on tumor number, size, macrovascular invasion, and extrahepatic metastasis by these imaging studies according to UICC-TNM classification.[3]

In addition to imaging related diagnoses, the 3 tumor markers are clinically used for HCC diagnosis in Japan; Alpha-fetoprotein (AFP), *Lens culinaris* agglutinin-reactive fraction of AFP (AFP-L3) and des-$\gamma$-carboxy prothrombin (DCP).

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Some studies have indicated that tumor markers are not only diagnostic tools for HCCs, but also can predict the degree of HCC malignancy.[4–6] However, the cutoff value of each tumor marker for predicting prognosis was still controversial, and there have been few reports which have investigated the importance of the expression patterns of multiple tumor markers for HCC on tumor characteristics and patient prognosis.

We hypothesized that the degree of HCC malignancy would progress according to the number of tumor markers that were elevated (AFP, AFP-L3, and DCP), and our aim was to investigate the influence of the expression pattern of these 3 tumor markers on the prognosis and clinicopathological parameters of HCC.

## PATIENTS AND METHODS

### Patients and Diagnosis

Between January 1999 and May 2009, 220 patients underwent a hepatectomy at Wakayama Medical University Hospital (WMUH), and were diagnosed with HCC by histological findings. Of these, 17 patients did not undergo a thorough histological examination because of preoperative treatment, 6 patients were diagnosed with combined hepatocellular and cholangiocarcinoma by histological findings, 1 patient was categorized as having a class C Child-Pugh score and 11 patients were lost to follow-up. These 35 patients were excluded from the present study, and the remaining 185 patients were enrolled in this retrospective study. All of the patients underwent several imaging studies, such as ultrasonography, dynamic CT, enhanced MRI and CT during arterial portography for diagnosis and staging of HCC before surgery. For the pathological diagnosis, histological grade of HCC was determined according to the Edmondson-Steiner classification.[7] The severity of liver disease (liver fibrosis staging and hepatitis activity grading) was also evaluated according to the criteria proposed by the Metavir cooperative study group.[8]

This retrospective study was conducted in accordance with the Declaration of Helsinki and the "ethical guidelines for clinical studies" of the ministry of Health, Labor and Welfare in Japan and also the guidelines of the Ethical Committee on Clinical Investigation of Wakayama Medical University Hospital.

### Tumor Markers

AFP, AFP-L3, and DCP were measured at the time of preoperative examinations. The serum AFP level was determined by chemiluminescent enzyme immunoassay (Siemens Immulite AFP IV, Mitsubishi Chemical Medience, Tokyo, Japan), the serum AFP-L3 level was expressed as a percentage of the total AFP (AFP-L3 / total AFP $\times$ 100) by lectin affinity electrophoresis coupled with antibody-affinity blotting (AFP-L3 Test Wako, Wako Pure Chemical Industries, Ltd., Osaka, Japan), and serum DCP was determined by chemiluminescent enzyme immunoassay (Lumipulse PIVKA-II Eisai, Eisai, Tokyo, Japan). The upper normal ranges of AFP, AFP-L3, and DCP in our institution were 20ng/mL, 10% and 40 mAU/mL, respectively. In this study, tumor markers that were higher than the upper normal range were defined as positive markers for HCC. Tumors were classified according to the number of positive tumor markers as "triple negative," "single positive," "double positive," or "triple positive."

### Treatment and Surveillance

At our institution, anatomical resections were basically planned for HCC if the liver function was well preserved and the estimated remnant liver volume met our criteria established previously.[9,10] In brief, our criteria is based on the formulations established by multiple regression analysis as follows: liver failure score = 164.8 − 0.58 × (ALB) − 1.07 × (HPT) + 0.062 × (AST) − 685 × (ICGK) − 3.57 × (OGTT.LI) + 0.074 × (RW) and logit score = 4.15 + 0.03 × (HA) − 0.16 × (remnantVol%), where ALB is serum albumin level (g/dL), HPT is hepaplastin test (%), AST is serum aspartate aminotrasnferase level (IU/L), ICGK is ICG clearance rate, OGTT.LI is blood glucose at 60 and 120 minutes after 75 g glucose oral intake, RW is the estimated resection liver volume, HA is serum hyaluronic acid level, and remnantVol% is the estimated remnant liver volume ratio. The logit score was specially adapted for the patient planned for lobectomy.[10] The value less than 25 of the liver failure score and the value less than 0 of the logit value were estimated as safe liver resection procedure.[9,10]

If any planned liver resection procedure did not meet our criteria, the patient underwent a treatment other than surgery. In this study, anatomical resections (segmental, sectional resection, and lobectomy or more) were performed in 119 patients. Nonanatomical resections were performed in 60 patients.

All of the patients were followed up by administering blood examinations and ultrasonography or abdominal contrast enhanced CT at WMUH every 2 to 3 months after surgery. When a recurrence of HCC was detected, patients received further treatment for HCC, such as repeat hepatectomy, ablation therapy, or transarterial chemoembolization (TACE). After treatment for recurrent lesions, the same surveillance was performed.

### Statistical Analysis

Dichotomous variables were compared using the $\chi^2$ test, and continuous variables were compared using *t* tests Recurrence free survival and disease-specific survival were analyzed using the Kaplan-Meier method and differences were analyzed using the logrank test.

To estimate the risk factors for recurrence and survival by Cox proportional hazards regression analysis, 18 variables were analyzed as follows: age, sex, presence of hepatitis B surface antigen (HBsAg), presence of hepatitis C antibody (HCVAb), Child-Pugh score, serum concentration of albumin, total bilirubin, aspartate aminotransferase (AST) and alanine aminotransferase (ALT), prothrombin time, indocyanine green dye retention rate at 15 minutes ($ICGR_{15}$), platelet count (PLT), number of tumors, maximum tumor size, fibrotic capsule formation and macrovascular invasion from the image findings, liver resection procedure, and triple positive tumor marker expression. In performing Cox proportional hazards regression analysis, continuous variables were converted into binary. The cutoff values were defined as follows; albumin, total bilirubin, and prothrombin time were basically based on the Child-Pugh score.[11] Tumor size was based on the UICC-TNM classification.[3] PLT was based on the value predicting cirrhosis. ICG $R_{15}$, AST, and ALT were based on the median values in this study. All analyses were performed using the SPSS software program ver.18.0 (SPSS Inc, Chicago, IL). $P < 0.05$ were considered to be significant.

## RESULTS

### Patient Characteristics and Tumor Markers

Among the 185 patients, there were 146 men and 39 women ranging in age from 33 to 89 years, with a mean ± SD of 67 ± 10 years. The median follow-up was 34 months (range, 2–112 months). During the follow-up period, 47 patients died of recurrence and progression of HCC, 5 patients died of hepatic failure, 3 patients died of gastrointestinal bleeding, 1 patient died of cerebral infarction, 1 patient died of esophageal carcinoma, and 1 patient died of a superior mesenteric artery embolism.

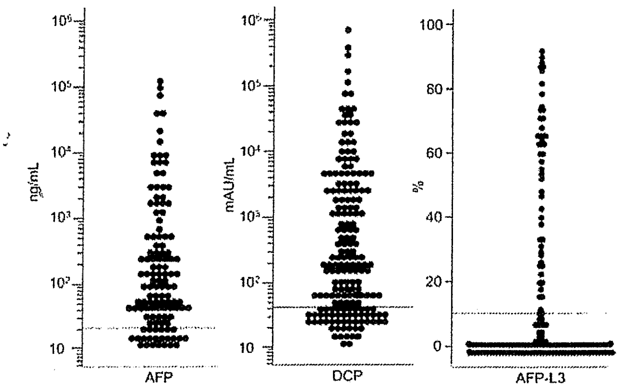


**FIGURE 1.** Distribution of the 3 tumor markers; alpha-fetoprotein (AFP), *Lens culinaris* agglutinin-reactive fraction of AFP (AFP-L3) and des-$\gamma$-carboxy prothrombin (DCP). The serum AFP and DCP levels are provided on a log scale. The dotted lines are the upper normal ranges of each tumor marker.

The distribution of serum AFP, AFP-L3, and DCP levels is presented as a dot plot (Fig. 1). The median values of serum AFP, AFP-L3, and DCP levels were 31.0 ng/mL, less than 0.5%, and 195 mAU/mL, respectively. Serum AFP, AFP-L3, and DCP levels over the upper normal ranges were observed in 100 patients (54.1%), 52 patients (28.1%), and 134 patients (72.4%), respectively. Table 1 shows the patients' background and clinical characteristics according to the elevation of each tumor marker. Positive expression of HCV Ab, prothrombin time, AST, and PLT were significantly higher in patients with elevated levels of AFP. The number of tumors, maximum tumor size, presence of macrovascular invasion in image findings, and anatomical liver resection were significantly higher in patients with elevated levels of AFP-L3. The PLT, maximum tumor size, presence of macrovascular invasion in image findings and anatomical liver resection were significantly higher in patients with elevated levels of DCP.

### Relationships Between Patient Backgrounds and the Number Of Positive Tumor Markers

According to qualitative evaluation of these 3 tumor markers, 75 patients were single positive [AFP (n = 16); DCP (n = 58); AFP-L3 (n = 1)], 47 patients were double positive (AFP and DCP (n = 35); AFP and AFP-L3 (n = 10); DCP and AFP-L3 (n = 2)], 39 patients were triple positive and 24 patients were triple negative. Among these 4 groups, no significant differences were observed in sex, age, HBsAg, HCVAb, Child-Pugh score, serum concentration of albumin, total bilirubin, AST and ALT, prothrombin time, $ICGR_{15}$, PLT, number of tumors, or fibrotic capsule formation (data not shown). On the contrary, a tumor size larger than 5 cm, the presence of macrovascular invasion, and anatomical liver resection were different among the 4 groups (Fig. 2).

### Relationships Between Patient Prognosis and the Number Of Positive Tumor Markers

The recurrence-free survival and disease-specific survival of the patients were also analyzed (Figs. 3 and 4). Recurrence-free

**TABLE 1.** Patient Backgrounds by Their Expression of the 3 Tumor Markers for HCC

| | AFP | | AFP-L3 | | DCP | |
|---|---|---|---|---|---|---|
| **Variable** | **<20 ng/mL** | **≥20 ng/mL** | **<10%** | **≥10%** | **<40mAU/mL** | **≥40mAU/mL** |
| Sex | | | | | | |
| Male/Female | 76/9 | 70/30 | 106/27 | 40/12 | 42/9 | 104/30 |
| Age | 68 ± 9 | 66 ± 10 | 67 ± 9 | 65 ± 11 | 67 ± 10 | 67 ± 10 |
| HbsAg | | | | | | |
| Negative/Positive | 74/11 | 78/22 | 112/21 | 40/12 | 41/10 | 111/23 |
| HCVAb | | | | | | |
| Negative/Positive | 48/37 | 35/65* | 61/72 | 22/30 | 18/33 | 65/69 |
| Child-Pugh score | | | | | | |
| Class A/Class B | 77/8 | 91/9 | 119/14 | 49/3 | 45/6 | 123/11 |
| Albumin (mg/dL) | 3.9 ± 0.5 | 3.9 ± 0.5 | 3.9 ± 0.5 | 3.9 ± 0.5 | 3.8 ± 0.5 | 4.0 ± 0.5 |
| Total bilirubin (mg/dL) | 0.9 ± 0.4 | 0.9 ± 0.4 | 0.9 ± 0.4 | 0.8 ± 0.3 | 1.0 ± 0.5 | 0.9 ± 0.3 |
| Prothrombin time (%) | 87.1 ± 12.8 | 82.9 ± 13.4* | 84.9 ± 12.7 | 84.6 ± 14.6 | 84.6 ± 11.1 | 84.9 ± 14.0 |
| $ICGR_{15}$ (%) | 13.2 ± 7.4 | 14.2 ± 7.9 | 14.3 ± 7.8 | 12.3 ± 7.1 | 15.6 ± 8.1 | 13.0 ± 7.4 |
| AST (IU/L) | 47.3 ± 23.5 | 58.9 ± 34.3* | 51.9 ± 30.1 | 57.8 ± 30.7 | 57.2 ± 29.4 | 52.2 ± 30.7 |
| ALT (IU/L) | 50.5 ± 32.6 | 57.2 ± 41.1 | 55.2 ± 40.4 | 51.2 ± 28.8 | 61.1 ± 38.9 | 51.4 ± 36.7 |
| PLT ($\times 10^4$/mm$^3$) | 18.9 ± 14.7 | 14.9 ± 6.0* | 16.9 ± 12.3 | 16.4 ± 6.9 | 13.7 ± 5.4 | 17.9 ± 12.4* |
| Number of tumors† | | | | | | |
| Single/Multiple | 60/25 | 64/36 | 95/38 | 29/23* | 36/15 | 88/46 |
| Tumor size† | | | | | | |
| ≤5 cm/>5 cm | 55/30 | 67/33 | 94/39 | 28/24* | 44/7 | 78/56* |
| Fibrotic capsule formation† | | | | | | |
| Absent/Present | 13/72 | 18/82 | 21/112 | 10/42 | 8/43 | 23/111 |
| Macro-vascular invasion† | | | | | | |
| Absent/Present | 72/13 | 72/28 | 116/17 | 28/24* | 47/4 | 97/37* |
| Liver resection | | | | | | |
| Anatomical/Nonanatomical | 54/31 | 65/35 | 77/56 | 42/10* | 22/29 | 97/37* |

Data are expressed as the means ± SD.
Anatomical resection indicates subsegmentectomy, segmentectomy, and lobectomy or more.
*$P < 0.05$ by $\chi^2$ test or $t$ test.
†Evaluated based on imaging findings.
ALT indicates alanine aminotransferase; AST, aspartate aminotransferase; HBsAg, hepatitis B virus surface antigen; HCVAb, anti-hepatitis C virus antibody; $ICGR_{15}$, indocyanine green dye retention rate at 15 min; PLT, platelet count.

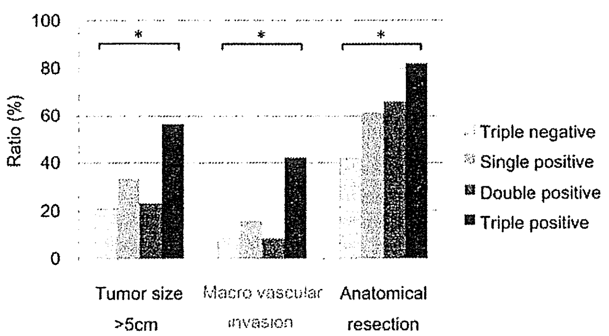


**FIGURE 2.** The relationships between clinical parameters and the positive patterns of the 3 tumor markers. *$P < 0.05$ by $\chi^2$ test.

survival rates at 2 years after hepatectomy were 19.4% in triple positive, 38.2% in double positive, 55.5% in single positive, and 50.7% in triple negative patients. Disease-specific survival rates at 5 years after hepatectomy were 35.9% in triple positive, 54.7% in double positive, 82.9% in single positive, and 62.8% in triple negative patients. Both recurrence-free and disease-specific survival curves revealed that the patients with triple positive tumor markers had a significantly worst prognosis.

## Factors Associated With Recurrence-free Survival Rates

The factors associated with recurrence-free survival were evaluated by univariate and multivariate analyses (Table 2). Univariate analysis revealed that the Child-Pugh score of class B, prothrombin time 80% or less, PLT less than 10 $\times$ $10^4$/mm$^3$, presence of multiple tumors, presence of macrovascular invasion from image findings and triple positive of tumor markers were significant variables. Multivariate analysis revealed that a Child-Pugh score of class B [relative risk (RR) 2.00, 95% confidence interval (95% CI) 1.03–3.87], the presence of multiple tumors (RR 2.53, 95% CI, 1.73–3.71), and triple positive markers for HCC (RR 1.78, 95% CI, 1.10–2.86) were independent risk factors for recurrence.

## Factors Associated With Disease-specific Survival Rates

The factors associated with disease-specific survival were also evaluated by univariate and multivariate analyses (Table 3). Univariate analysis revealed 5 significant variables; Child-Pugh score of class B, the presence of multiple tumors, tumor size larger than 5 cm, the presence of macrovascular invasion, and triple positive of tumor markers for HCC. In a multivariate analysis, 15 outcome events are generally needed to obtain 1 independent variable. As there were 48 survival outcomes in this study, the top 3 variables obtained by univariate analysis; triple positive tumor markers, the presence of macrovascular invasion, and the number of tumors, were entered into multivariate

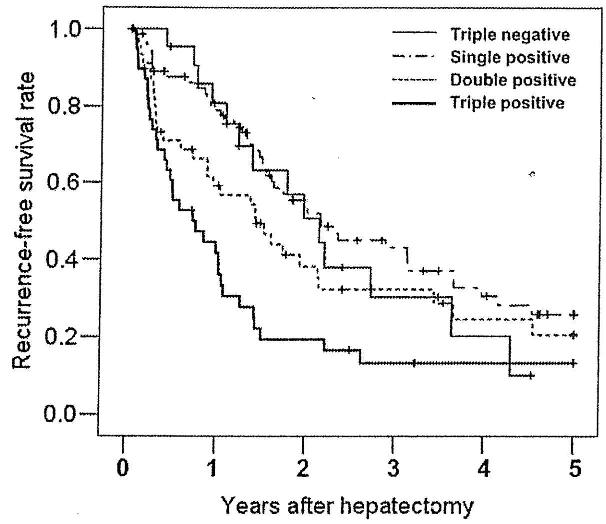


**FIGURE 3.** Recurrence-free survival curves among patients with different numbers of positive tumor markers. The triple positive group had a significantly shorter recurrence-free interval compared to the triple negative, single positive, and double positive groups (*P* values were 0.02, < 0.001, and 0.045, respectively).

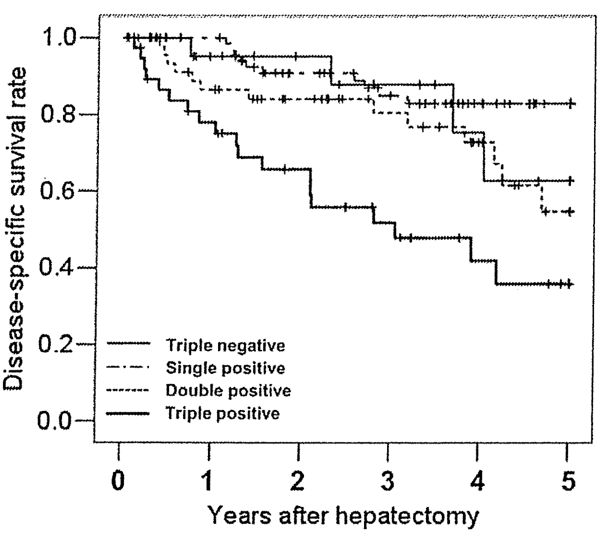


**FIGURE 4.** Disease-specific survival curves among patients with different numbers of positive tumor markers. The triple positive group had a significantly worse survival compared to the triple negative, single positive, and double positive groups (*P* values were 0.047, <0.001, and 0.02, respectively).

analysis, which revealed that the presence of multiple tumors (RR 2.38, 95% CI, 1.31–4.33) and triple positive markers for HCC (RR 2.41, 95% CI, 1.20–4.83) were independent risk factors for disease-specific survival.

## Comparison of Pathological Findings Among Number of Positive Tumor Markers

Pathological findings obtained after hepatectomy were compared among number of positive tumor markers (Fig. 5). The existence of microvascular invasion (triple negative, 16.7% (4/24); single positive, 29.3% (22/75); double positive, 46.8% (22/47); triple positive, 56.4% (22/39)) and an Edmondson-Steiner classification of III or IV [triple negative, 12.5% (3/24); single positive, 13.3% (10/75); double positive, 25.5% (12/47); triple positive, 35.9% (14/39)] were significantly different among these 4 groups.

## DISCUSSION

In this study, we showed that the patients who were triple positive for 3 tumor markers of HCC, namely AFP, AFP-L3, and DCP had shorter survival and more pathologically invasive characteristics.

Combinations of these 3 tumor markers are frequently used for detection of tumors in Japan and their usefulness has been reported previously.[12–14] Although previous articles showed the influence of each HCC marker on prognosis, most of these reports have examined the relationship between individual HCC tumor markers and prognosis.[4,15,16] There were few studies that investigated the significance of expression pattern of multiple tumor markers. One study reported that positivity for all 3 HCC tumor markers could predict the HCC patient's outcome.[17] However, their study included various treatment modalities such as hepatectomy, ablation therapy, and TACE. Their result might be biased by the treatment modalities and might be difficult to interpret the results for patients who underwent just a hepatectomy. In this study, we limited the patients to the hepatectomy population and clearly revealed the prognostic significance of triple positive tumor marker expression.

In this study, as shown in Table 1, the patients with AFP-L3 positive had a higher frequency of multiple tumors, large tumor size, and macrovascular invasion. Therefore, the following question would arise; how do the recurrence and survival curves compare between the triple positive patients and those who are just AFP-L3 positive? As previously described, the 5-year disease-specific survival rate and the 2-year recurrence-free survival rate of the triple positive patients were 35.9% and 19.5% in this study. Although those of the 13 patients with AFP-L3 positive but not triple positive tumor markers were 80.2% and 40%, respectively and revealed to be statistically different in 5-year disease-specific survival ($P = 0.03$), although a significant difference in 2-year recurrence-free survival was not seen ($P = 0.09$) (data not shown). Therefore, estimating just AFP-L3 would not be sufficient to evaluate the prognostic impact for HCC.

In this study, we defined cutoff values of the 3 tumor markers as above the upper normal limit. Previous reports have set the cutoff values as follows: (1) the median value, (2) the value calculated from receiver–operator characteristic (ROC) analysis, (3) the value of the upper or lower normal limit, and (4) values based on other reports.

First, in terms of the median value, we were able to calculate the median value of AFP and DCP. However, we could not evaluate the median value of AFP-L3, because most of the patients in this study had less than the minimum detectable limit for AFP-L3. Therefore, the median value could not be used for the cutoff value for the definition of positivity.

Second, ROC analysis in this study showed that the areas under the curve (AUCs) of AFP, AFP-L3, and DCP were 0.59, 0.58 and 0.55, respectively. Because the AUC values need to be at least more than 0.7 for significance discrimination, a cutoff value calculated from ROC analysis could not be used in this study.[18]

Moreover, referring to other reports is a good method for determining a cutoff value. However, different cutoff values were set in various reports, ranging from 20 to 400ng/mL for AFP[19–23], 10% to 15% for AFP-L3[23,24] and 40 to 100 mAU/mL for DCP.[13,25]

In addition, chronic hepatitis can affect serum AFP levels. However, a value of 16 ng/mL for serum AFP could distinguish HCC from chronic hepatitis.[26] Therefore, setting the cutoff value

**TABLE 2.** Univariate and Multivariate Analyses for Recurrence-free Survival in 185 Patients Undergoing Hepatectomy for Hepatocellular Carcinoma

| | | Univariate analysis | | Multivariate analysis | |
|---|---|---|---|---|---|
| **Variable** | **n** | **RR (95% CI)** | ***P*** | **RR (95% CI)** | ***P*** |
| Sex | | | | | |
| Female | 39 | 1 | | | |
| Male | 146 | 0.83 (0.55–1.27) | 0.39 | | |
| Age | | | | | |
| <68 years | 90 | 1 | | | |
| ≥68 years | 95 | 0.82 (0.57–1.17) | 0.27 | | |
| HBsAg | | | | | |
| Negative | 152 | 1 | | | |
| Positive | 33 | 1.21 (0.77–1.88) | 0.41 | | |
| HCVAb | | | | | |
| Negative | 83 | 1 | | | |
| Positive | 102 | 1.15 (0.80–1.65) | 0.45 | | |
| Child-Pugh score | | | | | |
| Class A | 168 | 1 | | | |
| Class B | 17 | 1.87 (1.05–3.34) | 0.03 | 2.00 (1.03–3.87) | 0.03 |
| Albumin | | | | | |
| >3.5 g/dL | 150 | 1 | | | |
| ≤3.5 g/dL | 35 | 1.39 (0.90–2.15) | 0.13 | | |
| Total bilirubin | | | | | |
| <1.0 mg/dL | 113 | 1 | | | |
| ≥1.0 mg/dL | 72 | 1.17 (0.82–1.68) | 0.38 | | |
| Prothrombin time | | | | | |
| >80% | 121 | 1 | | | |
| ≤80% | 64 | 1.52 (1.06–2.18) | 0.02 | 1.21 (0.81–1.83) | 0.34 |
| $ICGR_{15}$ | | | | | |
| <12.2% | 92 | 1 | | | |
| ≥12.2% | 93 | 1.33 (0.93–1.91) | 0.12 | | |
| AST | | | | | |
| <48 IU/L | 89 | 1 | | | |
| ≥48 IU/L | 96 | 1.34 (0.93–1.92) | 0.12 | | |
| ALT | | | | | |
| <45 IU/L | 92 | 1 | | | |
| ≥45 IU/L | 93 | 0.99 (0.70–1.42) | 0.97 | | |
| PLT | | | | | |
| $\geq 10 \times 10^4/mm^3$ | 159 | 1 | | | |
| $<10 \times 10^4/mm^3$ | 26 | 1.89 (1.12–3.00) | 0.007 | 1.56 (0.95–2.54) | 0.08 |
| Number of tumors* | | | | | |
| Single | 124 | 1 | | | |
| Multiple | 61 | 2.46 (1.69–3.56) | <0.0001 | 2.53 (1.73–3.71) | <0.0001 |
| Tumor size* | | | | | |
| ≤5 cm | 122 | 1 | | | |
| >5 cm | 63 | 1.31 (0.90–1.92) | 0.16 | | |
| Fibrotic capsule formation* | | | | | |
| Present | 154 | 1 | | | |
| Absent | 31 | 1.45 (0.92–2.28) | 0.11 | | |
| Macrovascular invasion* | | | | | |
| Absent | 144 | 1 | | | |
| Present | 41 | 1.65 (1.06–2.54) | 0.03 | 1.16 (0.71–1.90) | 0.56 |
| Liver surgery | | | | | |
| Nonanatomical | 66 | 1 | | | |
| Anatomical | 119 | 1.15 (0.80–1.66) | 0.46 | | |
| Tumor markers | | | | | |
| Nontriple positive | 146 | 1 | | | |
| Triple positive | 39 | 1.93 (1.27–2.93) | 0.002 | 1.78 (1.10–2.86) | 0.02 |

Triple positive patients are defined as those whose serum levels of all 3 tumor markers are above the normal range.

*Evaluated based on imaging findings.

ALT indicates serum alanine aminotransferase; AST, serum aspartate aminotransferase; CI, confidence interval; HBsAg, hepatitis B virus surface antigen; HCVAb, anti-hepatitis C virus antibody; $ICGR_{15}$, indocyanine green dye retention rate at 15 min; PLT, platelet count; RR: relative risk.

**TABLE 3.** Univariate and Multivariate Analyses for Disease-specific Survival in 185 Patients Undergoing Hepatectomy for Hepatocellular Carcinoma

| | | Univariate analysis | | Multivariate analysis | |
|---|---|---|---|---|---|
| Variable | n | RR (95% CI) | *P* | RR (95% CI) | *P* |
| Sex | | | | | |
| Female | 39 | 1 | | | |
| Male | 146 | 0.63 (0.34–1.19) | 0.16 | | |
| Age | | | | | |
| <68 years | 90 | 1 | | | |
| ≥68 years | 95 | 0.84 (0.46–1.51) | 0.55 | | |
| HBsAg | | | | | |
| Negative | 152 | 1 | | | |
| Positive | 33 | 1.67 (0.86–3.23) | 0.13 | | |
| HCVAb | | | | | |
| Negative | 83 | 1 | | | |
| Positive | 102 | 1.25 (0.68–2.29) | 0.47 | | |
| Child-Pugh score | | | | | |
| Class A | 168 | 1 | | | |
| Class B | 17 | 2.52 (1.17–5.42) | 0.02 | | |
| Albumin | | | | | |
| >3.5 g/dL | 150 | 1 | | | |
| ≤3.5 g/dL | 35 | 1.92 (0.99–3.72) | 0.054 | | |
| Total bilirubin | | | | | |
| <1.0 mg/dL | 113 | 1 | | | |
| ≥1.0 mg/dL | 72 | 0.78 (0.43–1.44) | 0.43 | | |
| Prothrombin time | | | | | |
| >80% | 121 | 1 | | | |
| ≤80% | 64 | 1.78 (0.99–3.21) | 0.056 | | |
| $ICGR_{15}$ | | | | | |
| <12.2% | 92 | 1 | | | |
| ≥12.2% | 93 | 0.84 (0.46–1.53) | 0.57 | | |
| AST | | | | | |
| <48 IU/L | 89 | 1 | | | |
| ≥48 IU/L | 96 | 1.35 (0.74–2.47) | 0.33 | | |
| ALT | | | | | |
| <45 IU/L | 92 | 1 | | | |
| ≥45 IU/L | 93 | 1.05 (0.58–1.89) | 0.87 | | |
| PLT | | | | | |
| $\geq 10 \times 10^4/mm^3$ | 159 | 1 | | | |
| $< 10 \times 10^4/mm^3$ | 26 | 1.83 (0.91–3.70) | 0.09 | | |
| Number of tumors* | | | | | |
| Single | 124 | 1 | | | |
| Multiple | 61 | 2.58 (1.43–4.66) | 0.002 | 2.38 (1.31–4.33) | 0.005 |
| Tumor size* | | | | | |
| ≤5 cm | 122 | 1 | | | |
| >5 cm | 63 | 2.30 (1.28–4.15) | 0.006 | | |
| Fibrotic capsule formation* | | | | | |
| Present | 154 | 1 | | | |
| Absent | 31 | 1.61 (0.80–3.26) | 0.18 | | |
| Macro-vascular invasion* | | | | | |
| Absent | 144 | 1 | | | |
| Present | 41 | 2.92 (1.55–5.50) | 0.0009 | 1.67 (0.80–3.48) | 0.17 |
| Liver surgery | | | | | |
| Nonanatomical | 66 | 1 | | | |
| Anatomical | 119 | 1.57 (0.82–2.99) | 0.17 | | |
| Tumor markers | | | | | |
| Nontriple positive | 146 | 1 | | | |
| Triple positive | 39 | 3.10 (1.69–5.67) | 0.0002 | 2.41 (1.20–4.83) | 0.01 |

Triple positive patients are defined as those whose serum levels of all 3 tumor markers are above the normal range.

*Evaluated based on imaging findings.

ALT indicates serum alanine aminotransferase; AST, serum aspartate aminotransferase; CI, confidence interval; HBsAg, hepatitis B virus surface antigen; HCVAb, anti-hepatitis C virus antibody; $ICGR_{15}$, indocyanine green dye retention rate at 15 min; PLT, platelet count; RR, relative risk.

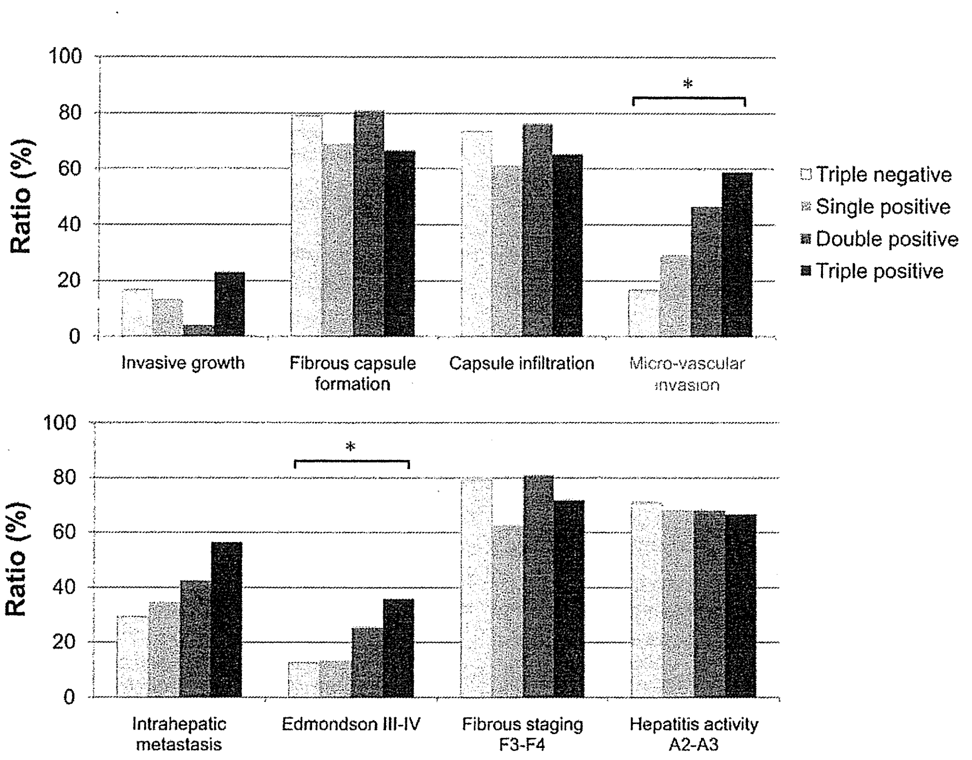


**FIGURE 5.** The relationships between pathological parameters and the positive expression pattern of the 3 tumor markers. $^{*}P < 0.05$ by $\chi^2$ test.

as the upper normal limit (20 ng/mL for AFP) was appropriate for predicting the prognosis of HCC.

For the reasons stated earlier, the cutoff values of these 3 tumor markers were defined as upper normal limits in this study.

The type of surgical procedure selected was also considered to affect the prognosis, and anatomical resection was reported to have a beneficial effect on prognosis.[27] Although in this study anatomical resection was more common in the "triple positive" group, the prognosis was significantly worse. This might indicate that "triple positive" HCC has a higher degree of tumor malignancy.

This study also revealed pathological differences among the number of positive tumor markers. Indeed, several studies revealed that high serum levels of tumor markers were predictive of portal vein invasion.[4,28] In addition to the previous study, our pathological findings revealed that there is increasing invasiveness and poorer differentiation characteristics in HCC according to the number of positive tumor markers.

In general, vascular invasion has a prognostic impact. However, our result in this study failed to reveal an independent risk factor. In this study, the factor of macrovascular invasion was evaluated by imaging studies. Therefore, microvascular invasions that could not be detected by imaging studies were entered into absent macrovascular invasions in Tables 2 and 3. Therefore, the statistical power of discrimination might be diminished. Furthermore, the triple positive tumor marker, which revealed to represent tumor invasiveness including microvascular invasion, was a stronger factor than the factor of macrovascular invasion evaluated by imaging studies. In this point, the positivity of 3 tumor markers would reflect and involve the prognostic impact of vascular invasion.

In the clinic, our results would be informative for selecting the most appropriate therapeutic modality. If their liver function was well preserved, the patients with triple positive tumor marker might avoid locogerional ablation therapy because of its pathological invasiveness. However, anatomical resection was performed in a large number of triple positive patients in this study and the recurrence rate was also high. Therefore, these patients might be candidates for adjuvant chemotherapies. Otherwise, these patients might be recommended for liver transplantation.

Recently, some other newly biological markers such as Glypican-3 has been identified for HCC.[29] Although these biomarkers are not currently in clinical use, these biomarkers identified by molecular biological methods would reveal biological function of HCC in further analyses and might change the importance of triple positive of AFP, AFP-L3, and DCP as a prognostic marker for patients with HCC after hepatectomy.

In conclusion, a triple positive status for the 3 tumor markers of HCC was an independent risk factor for recurrence and survival, and indicated an increasing presence of vascular invasion and poor differentiation in pathological findings.

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ORIGINAL ARTICLE

# Impact of nodal involvement on surgical outcomes of intrahepatic cholangiocarcinoma: a multicenter analysis by the Study Group for Hepatic Surgery of the Japanese Society of Hepato-Biliary-Pancreatic Surgery

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**Abstract**

*Background/purpose* The aim of this study was to clarify the prognostic factors of intrahepatic cholangiocarcinoma (ICC) following hepatectomy and to examine the impact of lymph node metastasis on survival. This study was therefore carried out as a Project Study of the Japanese Society of Hepato-Biliary-Pancreatic Surgery.

*Methods* Three hundred and forty-one patients who underwent hepatectomy for ICC between 1995 and 2004 at the 9 institutions of the Medical University Hospitals were analyzed retrospectively. Multivariate regression analyses and a Kaplan–Meyer analysis were performed to identify prognostic factors.

*Results* Pathological lymph node metastasis was one of the significant factors affecting overall survival (hazard ratio 2.10, $p < 0.001$) based on the multivariate analysis. Among the patients who underwent extended lymphadenectomy beyond the hepatoduodenal ligament, the median survival of 121 patients with nodal involvement was 12.2 months. Only seven patients with nodal involvement have survived for more than 4 years.

*Conclusions* In the present study, preoperative carbohydrate antigen (CA) 19-9, intrahepatic metastasis, and nodal involvement were the significant independent predictors of poor prognosis by multivariate analysis. Further prospective studies may thus be needed to confirm these findings, because this study has a limitation in that it was a retrospective study with multicenter data collection.

**Keywords** Overall survival · Lymph node metastasis · Extended lymph node dissection · Surgical margin · Intrahepatic metastasis · CA19-9

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## Introduction

Intrahepatic cholangiocarcinoma (ICC) is defined as that localized in the liver, and it usually does not present with obstructive jaundice. Although ICC is the second most common primary hepatic tumor, following hepatocellular carcinoma (HCC), a recent survey of 18,213 newly diagnosed primary liver cancer patients in Japan demonstrated that only 4.1% of patients had ICC, compared with 94.2% who presented with HCC [1]. ICC has been considered to be a highly malignant neoplasm, because it is frequently associated with lymph node involvement, intrahepatic metastasis, peritoneal dissemination, and/or infiltration into the bile duct and portal vein in the hepatic hilus [2, 3]. The main obstacle to the curative treatment of ICC is the time of diagnosis, because a high percentage of tumors are already at an unresectable stage when the cancer is diagnosed [4–6]. Only the surgical resection of ICC has been shown to improve long-term survival [7–9]. Unfortunately, the resectability rate is low, and varies in the literature from 34 to 62% [2–6]. Moreover, for many patients, the prognosis after aggressive liver resection still remains unsatisfactory. However, some authors have reported that by using aggressive surgical approaches, even patients with advanced ICC can expect prolonged survival following a curative hepatic resection [7–11]. Several clinicopathologic factors such as tumor markers, macroscopic type, tumor size, tumor number, histological differentiation status, surgical margins, and lymph node metastasis, have been reported to be significant prognostic factors for survival [1–11].

However, it still remains controversial as to whether ICC patients with lymph node metastasis can obtain any survival benefit from surgery. Lymph node dissection for ICC remains a controversial issue [2]. Although it has been suggested that the standard surgical procedure for ICC is hepatectomy combined with extensive nodal dissection, some centers do not support the routine application of lymph node dissection [7]. Selective lymphadenectomy and limited application of lymph node dissection [8] have been reported for other institutions. In other words, only suspicious lymph nodes were submitted for frozen section examination before proceeding to liver resection in these patients; however, other authors advocate lymph node dissection only in the context of extended resection and not as far as standard resection is concerned. There is currently no evidence concerning the efficacy of extended lymphadenectomy in ICC patients. By carrying out a retrospective analysis of consecutive patients at 9 high-volume centers, we investigated the outcomes of ICC patients with lymph node metastasis following hepatic resection, and analyzed the prognostic factors affecting patient survival. The distribution of the lymph node metastases and the influence of the extent of lymph node dissection on survival were examined in order to clarify the indications for the surgical treatment of ICC with lymph node metastases.

## Patients and methods

Three hundred and forty-one consecutive patients with ICC who underwent hepatectomy between 1995 and 2004 at the 9 institutions of the Medical University Hospitals were retrospectively analyzed. The 9 institutions selected for this project study are high-volume centers in Japan, and at these centers, the standard surgical procedure for ICC is hepatectomy combined with dissection of primary lymph nodes. ICC was defined as adenocarcinoma arising from the second order or more distal branches of the intrahepatic bile ducts [7, 8]. Patients with combined HCC and cholangiocarcinoma or bile duct cystadenocarcinoma were excluded from this study cohort. The patient demographics, medical history, symptoms, laboratory findings, clinicopathological features, surgical management, and hospital morbidity and mortality were reviewed. All patients underwent routine liver function tests: bilirubin, albumin, prothrombin time, alkaline phosphatase, transaminase, hepatitis B surface antigen (HBsAg), antibody to hepatitis C virus (anti-HCV), and the indocyanine green test; also, their blood cell counts, serum creatinine levels, results of serum carcinoembryonic antigen (CEA) and carbohydrate antigen (CA) 19-9 assays, and the results of chest radiography before hepatectomy were all examined. The preoperative diagnosis was based on a combination of imaging data including computed tomography (CT), ultrasonography (US), and/or magnetic resonance imaging (MRI).

Lymph node dissection was not performed uniformly in all patients because of the multicenter retrospective nature of the study. Of the 341 patients, 111 patients did not undergo lymph node dissection (68 patients) or lymph node sampling along the hepatoduodenal ligament lymph nodes (43 patients), and these 111 patients did not demonstrate lymph node metastases in the preoperative imaging studies. Our lymph node sampling indicated that only suspicious lymph nodes were submitted for frozen section examinations. The lymph nodes were retrieved in 228 patients with extended lymph node dissection, which means the lymphadenectomy extended beyond the hepatoduodenal ligament nodes, including lymph nodes along the common hepatic artery and retropancreas and/or more distal lymph nodes.

Macroscopically, the number of tumors, tumor size, macroscopic type, and surgical margins of the tumors were evaluated. The macroscopic type of ICC was categorized into the following types: mass-forming (MF), periductal infiltration (PI), and intraductal growth (IG) types, according to the Liver Cancer Study Group of Japan [12]. The microscopic characteristics evaluated were: histological type, level of differentiation, margin status, and the presence of lymph node metastases. We categorized the distribution of lymph node metastases as: hepatoduodenal ligament lymph nodes (n1), lymph nodes along the common hepatic artery and retropancreatic lymph nodes (n2), lymph nodes along the celiac axis and left gastric artery (n3), and paraaortic lymph nodes (n4).

Hepatectomy was defined as the resection of one or more liver segments by Couinaud's definition [13]. Right hepatectomy (subsegments 5 + 6 + 7 + 8), right trisectionectomy (1 + 4 + 5 + 6 + 7 + 8), left hepatectomy (2 + 3 + 4), left trisectionectomy (1 + 2 + 3 + 4 + 5 + 8), central hepatectomy (4 + 5 + 8), right posterior sectionectomy (6 + 7), right anterior sectionectomy (5 + 8), and left lateral sectionectomy have all been defined as hepatectomies according to Couinaud's classification. After resection, all patients underwent regular follow-ups over a 5-year period (or until death) with clinical examinations and blood chemistry evaluations. The patients were screened for CEA and CA19-9 to identify tumor recurrence after operation, and underwent CT scan and/or MRI. Palliative adjuvant chemotherapy with various protocols, usually based on 5-fluorouracil (FU), cisplatin, or gemcitabine without tumor relapse after surgery, was performed in only 68 of the 341 patients. Survival was calculated in terms of the resection margin, tumor nodules, tumor size, status of lymph node metastasis, blood loss, periductal invasion, tumor differentiation, biliary tract reconstruction, lymph node dissection, and adjuvant chemotherapy following the operation. The width of the resection margin was measured in patients with an R0-resection. Disease-free survival was calculated from the date of surgery to the date of recurrence, and the overall survival was calculated from the date of surgery. Survival was analyzed using the Log-rank test.

Surgical blood loss and serum CA19-9 were divided into 2 categories according to the cutoff median value, because receiver operating characteristic (ROC) analysis of surgical blood loss could not be applied in this multivariate analysis. All continuous data are shown as median values with a range of minimum and maximum values. The relationships between categorical variables were tested using $\chi^2$ analysis, and continuous data were tested by the Mann–Whitney $U$-test. Patient survival was calculated using the Kaplan–Meier method, and comparisons of survival curves were made with the Log-rank test. Potential predictors of survival were evaluated in multivariate Cox proportional hazards regression models. $p$ values of <0.05 were considered to be statistically significant. Data were analyzed using the SPSS version 13.0 software program (Statistical Package for Social Science, Chicago, IL, USA).

## Results

### Background of patients and preoperative clinical findings

The demographic and preoperative clinical data of the 341 patients with ICC who underwent liver resection are shown in Table 1. There were 217 male and 124 female patients, with a mean age of 64 years (range 30–84 years). Causes of liver disease were hepatitis B virus in 32 patients (9.4%) and hepatitis C virus in 64 patients (18.8%). Among these 341 patients who underwent a liver resection, the MF type was present in 183 (54%), the PI + MF type in 100 (29%), the PI type in 29 (9%), and the IG type in 22 (7%) patients. The tumor was identified as a well-differentiated adenocarcinoma in 56 of the 270 patients for whom differentiation was noted (21%), moderately differentiated type in 177 (66%), and poorly differentiated type in 37 (14%). Preoperative blood chemical data showed abnormal values for serum CA19-9 (median 135 U/ml, range 0.1–120,000 U/ml) and serum alkaline phosphatase (median 401 IU/l, range 25–5,335 IU/l) (Table 2).

### Hepatectomy procedures and intraoperative findings

Tumor removal was achieved by right hepatectomy in 90 patients, right trisectionectomy in 18, left hepatectomy in 118, left trisectionectomy in 25, central hepatectomy (i.e., Couinaud segments 4, 5, and 8) in 10, right anterior sectionectomy in 7, right posterior sectionectomy in 9, left lateral sectionectomy in 15, segmentectomy in 14, and nonanatomic liver resection in 21 patients (Table 3). There were 261 extended resections (>2 liver sections; 76.5% of

**Table 1** Background of the patients (1995–2004, $n = 341$)

| | |
|---|---|
| Age (years)[a] | 64 (30–84) |
| Gender (male/female) | 217/124 |
| Child–Pugh class | |
| A/B/C | 261/28/0 |
| Background liver | |
| Normal/chronic hepatitis/cirrhosis | 213/48/28 |
| Viral infection | |
| B/C/B and C/no viral infection | 23/55/9/254 |
| History of blood transfusion | |
| Yes/no | 25/236 |
| Macroscopic tumor type | |
| MF/PI + MF/PI/IG/MF + IG | 183/100/29/22/7 |
| Histological differentiation type | |
| Well/moderately/poorly | 56/177/37 |

*MF* mass-forming type, *PI* periductal-infiltrating type, *IG* intraductal-growth type

[a] Values are expressed as medians (ranges)

the 341 patients). The associated biliary procedures included resection of the biliary confluence and extrahepatic bile duct with Roux-en-Y hepatico-jejunostomy in 195 patients (57.1%), and vascular reconstructions were required in 53 patients (15.5%). Vascular reconstruction consisted of portal vein anastomosis in 29 patients, hepatic artery anastomosis in 5 patients, and inferior vena cava anastomosis (including the hepatic vein) in 19 patients because of the possibility of a curative liver resection for ICC. The median blood loss was 1,400 ml (range 50–10,350 ml) and the median duration of surgery was 435 min (range 90–1,140 min). Regarding the tumor findings during surgery, the median tumor diameter (in cases of multifocal tumors, the diameter of the largest nodule) was 45 mm (range 7–180 mm), and solitary tumors were found in 242 patients (70.9%).

Postoperative mortality and morbidity

Postoperative mortality (within 30 days or during the initial hospitalization) occurred in 11 of the resected patients (3.2%). A total of 117 cases of postoperative morbidity occurred in the 341 patients (34.3%), including bile leakage ($n = 44$), surgical site infection ($n = 42$), pleural effusion ($n = 30$), intra-abdominal fluid collection ($n = 18$), hyperbilirubinemia ($n = 16$), and liver failure ($n = 13$) (Table 4). None of the complications were correlated with either the type of liver resection or the extension of the resection to include major vessels and/or adjacent structures, nor was there any direct correlation with the extent of lymph node metastasis. The median hospitalization period was 29 days (range 5–744 days).

**Table 2** Preoperative blood chemical data (1995–2004, $n = 341$)

| | |
|---|---|
| Total bilirubin (mg/dl) | 0.7 (0.1–22.7) |
| Albumin (mg/dl) | 3.9 (2.5–5.2) |
| ICG R15 (%) | 9.6 (1.0–39.0) |
| Prothrombin time (%) | 94 (33–150) |
| CEA (ng/ml) | 3.3 (0.6–3,570) |
| CA19-9 (U/ml) | 135 (0.1–120,000) |
| AST (IU/l) | 33 (9–287) |
| ALT (IU/l) | 31 (6–624) |
| Alkaline phosphatase (IU/l) | 401 (25–5,335) |
| Platelets ($10^4/\mu l$) | 22 (6–57) |

Values are expressed as medians (ranges)

*ICG R15* indocyanine green retention rate at 15 min, *CEA* carcinoembryonic antigen, *CA 19-9* carbohydrate antigen 19-9, *AST* aspartate aminotransferase, *ALT* alanine aminotransferase

**Table 4** Operative morbidity and mortality

| Complication | Number (%) |
|---|---|
| Bile leakage | 44 (12.9%) |
| Surgical site infection | 42 (12.3%) |
| Pleural effusion | 30 (8.8%) |
| Intraabdominal fluid collection | 18 (5.3%) |
| Hyperbilirubinemia | 16 (4.7%) |
| Liver failure | 13 (3.8%) |
| Intraabdominal bleeding | 7 (2.1%) |
| Gastrointestinal bleeding | 6 (1.8%) |
| Pneumonia | 2 (0.6%) |
| Others | 2 (0.6%) |
| All complications | 191 |
| Perioperative deaths | 11 (3.2%) |

Complication rate 128/341 (37.5%)

**Table 3** Hepatectomy procedures

| | Resected segment (Couinaud's classification) [13] | Number | Subtotal |
|---|---|---|---|
| Hemihepatectomy or more | | | |
| Right hepatectomy[a] | 5, 6, 7, 8 | 90 | 261 (76.5%) |
| Right trisectionectomy | 4, 5, 6, 7, 8 | 18 | |
| Left hepatectomy[a] | 2, 3, 4 | 118 | |
| Left trisectionectomy | 2, 3, 4, 5, 8 | 25 | |
| Central hepatectomy | 4, 5, 8 | 10 | |
| Sectionectomy | | | |
| Right anterior sectionectomy | 5, 8 | 7 | 31 (9.1%) |
| Right posterior sectionectomy | 6, 7 | 9 | |
| Left lateral sectionectomy | 2, 3 | 15 | |
| Segmentectomy | | | 14 |
| Partial resection | | | 21 |
| Others[b] | | | 14 |
| Total | | | 341 |

Biliary tract reconstructions were conducted in 195 patients and vascular reconstructions were required in 53 patients

[a] Including extended hepatectomy

[b] Patients with multiple liver resections for multifocal tumors

Survival and prognostic factors

All patients were followed for more than 5 years (range 5.0–10.5 years). The overall median survival was 20.0 months, with cumulative 3- and 5-year rates of 36.4 and 29.2%, respectively. A total of 75 patients (22.0%) survived for more than 5 years. The median recurrence-free survival was 10.1 months, with 3- and 5-year rates of 29.5 and 25.1%, respectively. According to the macroscopic tumor type, 15/22 patients (68.2%) with IG tumors survived for more than 5 years and the rate of lymph node metastases was 0% in the IG tumors. The results of the univariate and multivariate analyses are summarized in Table 5. Univariate analysis demonstrated that significant prognostic factors for poorer survival were a high preoperative serum CA19-9 level (>135 U/ml) ($p < 0.001$), the presence of intrahepatic metastasis ($p < 0.001$), tumor size greater than 5 cm ($p < 0.001$), massive bleeding of more than 1,400 ml during surgery ($p = 0.005$), the presence of positive surgical margins ($p < 0.001$), macroscopic tumor type ($p < 0.001$), histological lymph node metastasis ($p < 0.001$), and histological differentiation ($p = 0.016$). Gender, the presence of liver cirrhosis, the need for biliary tract reconstruction, extended lymph node dissection beyond the hepatoduodenal ligament, and the use of adjuvant chemotherapy after the surgery did not significantly correlate with patient survival following liver resection. We selected the following parameters for the multivariate analysis; tumor size, the number of tumors, the serum CA19-9 level, pathological lymph node metastasis, blood loss, surgical margin, histological differentiation, and macroscopic tumor type, because these parameters had $p$ values of less than 0.05 in the univariate analysis. On multivariate Cox proportional hazards regression analysis, significant factors affecting overall survival were a high preoperative serum CA19-9 level (>135 U/ml) (hazard ratio 2.07, $p < 0.001$), the presence of intrahepatic metastasis (hazard ratio 2.00, $p < 0.001$), pathological lymph node metastasis (hazard ratio 2.10, $p < 0.001$), and a positive surgical margin (hazard ratio 1.81, $p = 0.006$). Among the patients who underwent extended lymphadenectomy beyond the hepatoduodenal ligament, the patient group with node-negative lymph node metastasis showed significantly better survival than the patient group with nodal involvement (Fig. 1) (Log-rank test, $p < 0.001$). The median survival of the 121 patients with nodal involvement was 12.2 months.

Predictive risk factors for lymph node metastases

The results of the univariate and multivariate analyses, in terms of risk factors for lymph node metastases are summarized in Table 6. Univariate analysis demonstrated that the significant risk factors for nodal involvement were a high preoperative serum CA19-9 level (>135 U/ml) ($p < 0.001$), macroscopic tumor type ($p < 0.001$), the presence of intrahepatic metastasis ($p = 0.017$), and histological differentiation ($p = 0.006$). Onmultivariate logistic regression analysis, the only significant factor to predict lymph node metastasis was a high preoperative serum CA19-9 level (>135 U/ml) (odds ratio 5.34, $p < 0.001$).

Overall survival was not related to either the macroscopic type (Fig. 2) or the histological differentiation type (Fig. 3) in patients with histologically positive lymph node metastases, as indicated by the Kaplan–Meier survival analysis. Only seven patients with nodal involvement have survived for more than 4 years, including the patient with paraaortic lymph node metastases (Table 7). As for the adjuvant chemotherapy, 5-FU was used in 2 patients; namely, patient No. 2 patient received it for 2 years, while patient No.4 received it for 6 months.

## Discussion

Epidemiologic evidence has suggested that hepatitis C viral infection, obesity-related nonalcoholic fatty liver disease, and nonalcoholic hepatic cirrhosis, all of which are rising in incidence, are risk factors for ICC [1–5]. Recently, infection with HCV or HBV has been suggested to be a risk factor for ICC [14]. A multivariate analysis found anti-HCV positivity, but not HBsAg positivity, to be associated with ICC [14]. A prospective cohort study from Japan showed that 2.3% of 600 patients with HCV-related cirrhosis developed ICC during a mean follow-up of 7.2 years. The risk of developing ICC among patients with HCV-related-cirrhosis was 1,000 times higher than that in the general population [15]. According to our data, 64 patients (18.8%) suffered from hepatitis C viral infection.

Only surgical resection of the liver has allowed patients with ICC to achieve long-term survival; however, the long-term survival of these patients remains unsatisfactory, and the prognosis of ICC patients is generally poor [1–11]. Surgery for ICC often requires extended hepatic resection. In our study, we performed major hepatectomy in 76.5% of the patients, with an extrahepatic bile duct resection being necessary in 42.8% of the patients. Both a vascular resection and reconstruction were required in 15.5% of the patients, with macroscopic tumor invasion indicating more advanced tumors and more difficult resections. In this respect, the need for a resection of the hilar bifurcation was based on oncologic factors rather than the technical aspects of the right or left hepatic duct after the resection of the ICC located close to these structures. Although both surgical techniques and postoperative management have been improving, extended hepatectomy still entails significant

**Table 5** Univariate and multivariate analyses of prognostic factors for overall survival following hepatectomy

| Variables | Number | Univariate analysis | | Multivariate analysis | | | |
|---|---|---|---|---|---|---|---|
| | | Survival (%) | | *p* value[a] | Hazard ratio | 95% Confidence interval (CI) | *p* value[c] |
| | | 3 year | 5 year | | | | |
| Gender | | | | | | | |
| Male | 217 | 34.7 | 26.9 | | | | |
| Female | 124 | 39.5 | 33.1 | 0.257 | | | |
| Liver cirrhosis | | | | | | | |
| No | 310 | 36.8 | 29.0 | | | | |
| Yes | 13 | 48.4 | 48.4 | 0.431 | | | |
| Serum CA 19-9 (U/ml) | | | | | | | |
| ≤135 | 158 | 59.1 | 50.9 | | 1 | | |
| >135 | 154 | 21.3 | 13.3 | <0.001 | 2.07 | 1.41–3.05 | <0.001 |
| Tumor size (cm) | | | | | | | |
| ≤5 cm | 204 | 42.9 | 35.9 | | 1 | | |
| >5 cm | 130 | 25.1 | 17.2 | <0.001 | 1.11 | 0.79–1.54 | 0.566 |
| Number of tumors | | | | | | | |
| Single | 242 | 43.9 | 36.4 | | 1 | | |
| Multiple | 97 | 17.5 | 9.2 | <0.001 | 2.00 | 1.39–2.90 | <0.001 |
| Pathological lymph node metastasis | | | | | | | |
| Negative | 141 | 52.9 | 46.4 | | 1 | | |
| Positive | 139 | 13.6 | 7.0 | <0.001 | 2.10 | 1.41–3.00 | <0.001 |
| Blood loss (ml) | | | | | | | |
| ≤1,400 | 153 | 43.4 | 35.0 | | 1 | | |
| >1,400 | 156 | 30.4 | 23.5 | 0.005 | 1.36 | 0.98–1.89 | 0.066 |
| Surgical margin | | | | | | | |
| Negative | 272 | 41.7 | 33.9 | | 1 | | |
| Positive | 47 | 12.4 | 7.4 | <0.001 | 1.81 | 1.18–2.78 | 0.006 |
| Macroscopic tumor type | | | | | | | |
| IG | 22 | 90.7 | 79.3 | <0.001 | 1 | | |
| MF | 191 | 38.6 | 30.1 | | 3.29 | 0.98–10.98 | 0.053 |
| PI, PI + MF | 129 | 24.1 | 19.4 | | 3.03 | 0.90–10.26 | 0.075 |
| Biliary tract reconstruction | | | | | | | |
| No | 146 | 32.8 | 26.1 | | | | |
| Yes | 195 | 41.3 | 33.3 | 0.090 | | | |
| Extended lymph node dissection[b] | | | | | | | |
| No | 111 | 39.6 | 32.9 | | | | |
| Yes | 228 | 42.7 | 34.1 | 0.254 | | | |
| Histological differentiation | | | | | | | |
| Well | 62 | 47.4 | 39.8 | | 1 | | |
| Moderately | 210 | 35.8 | 29.1 | | 0.77 | 0.50–1.19 | 0.239 |
| Poorly | 56 | 30.6 | 22.4 | 0.016 | 1.05 | 0.59–1.90 | 0.864 |
| Adjuvant chemotherapy following operation | | | | | | | |
| No | 270 | 37.1 | 28.7 | | | | |
| Yes | 68 | 33.8 | 32.1 | 0.451 | | | |

*CI* confidence interval

[a] Log-rank test

[b] Lymph node dissection beyond the hepatoduodenal ligament nodes, including lymph nodes along the common hepatic artery and retropancreas and/or more distal lymph nodes

[c] Cox regression analysis

morbidity and mortality. Most surgical series have reported a mortality rate lower than 5%, but the complication rate is still high, and varies from 20 to 50% [1–11]. In our patients, the mortality and morbidity rates were 3.2 and 34.3%, which are average values in comparing with another reports.

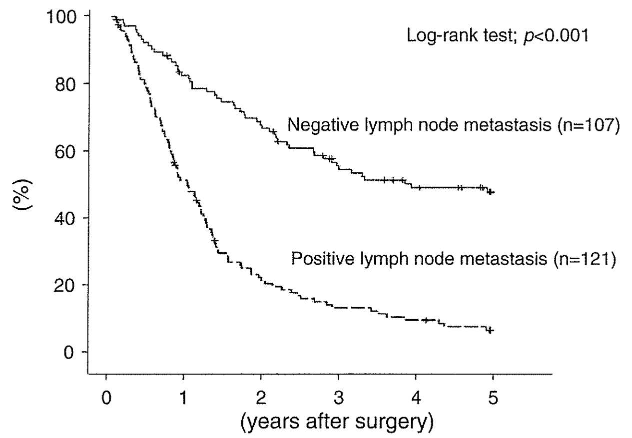


**Fig. 1** Overall survival for the patients following extended lymphadenectomy beyond the hepatoduodenal ligament. The presence of lymph node metastasis was a significantly poor prognostic factor for patients with intrahepatic cholangiocarcinoma (ICC)

In the present study, the macroscopic type of tumor proposed by the Japan Liver Cancer Study Group in 2008 [12] was shown to be associated with the prognosis of ICC. The frequency of lymph node involvement is significantly related to the macroscopic type: IG-type tumors have a lower frequency of lymph node involvement than the other macroscopic types [9]. Ohtsuka et al. [16] reported that all nine of their patients with IG-type cancer remained alive for periods ranging from 8 to 72 months; eight patients had no recurrence, and one experienced recurrence. In the present study, all 22 patients with IG-type tumors were found to be free of lymph node involvement, and 15 of these patients (68.2%) remained alive for more than 5 years after surgery, without recurrence. The differences in the rate of lymph node involvement for the MF and PI types have been less clear [4]. In our study, the rate of lymph node metastasis was significantly related to the histological differentiation type: well-differentiated-type tumors had a lower frequency of lymph node metastases than the moderately and poorly differentiated types. Although the differences in the rate of lymph node involvement according to the histological differentiation type are still unclear, well-differentiated-type tumors had a significantly lower rate of lymph node metastases in our analysis.

**Table 6** Univariate and multivariate analyses of risk factors for lymph node metastases

| Variables | Number | Univariate analysis | | Multivariate analysis | | |
|---|---|---|---|---|---|---|
| | | Lymph node metastasis negative/positive | *p* value[a] | Odds ratio | 95% CI | *p* value[b] |
| Tumor size | | | | | | |
| ≤5 cm | 132 | 64/68 | 0.358 | | | |
| >5 cm | 90 | 38/52 | | | | |
| Number of tumors | | | | | | |
| Single | 172 | 88/84 | 0.017 | 1 | | |
| Multiple | 55 | 18/37 | | 1.83 | 0.88–3.82 | 0.109 |
| Serum CA19-9 (U/ml) | | | | | | |
| ≤135 | 98 | 67/31 | <0.001 | 1 | | |
| >135 | 123 | 36/87 | | 5.34 | 2.77–10.27 | <0.001 |
| Macroscopic tumor type | | | | | | |
| IG | 13 | 13/0 | <0.001 | –[c] | | |
| MF | 104 | 49/55 | | 1 | | |
| PI, PI + MF | 112 | 45/67 | | 0.98 | 0.51–1.89 | 0.946 |
| Histological differentiation | | | | | | |
| Well | 37 | 26/11 | 0.006 | 1 | | |
| Moderately | 148 | 60/88 | | 2.51 | 0.98–6.47 | 0.056 |
| Poorly | 30 | 11/19 | | 2.32 | 0.70–7.69 | 0.171 |

Patients with extended lymphadenectomy, $n = 228$

[a] $\chi^2$ analysis

[b] Logistic regression analysis

[c] Not detected because of no lymph node metastasis

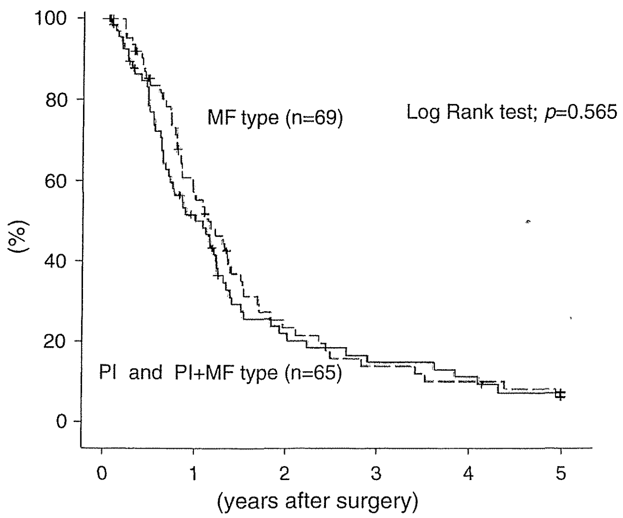


**Fig. 2** Overall survival for the patients with positive lymph node metastases based on the macroscopic tumor type. Among the patients diagnosed as being positive for lymph node metastases there were no significant differences in the survival rates between the macroscopic tumor types. *MF* Mass-forming type, *PI* periductal-infiltration type

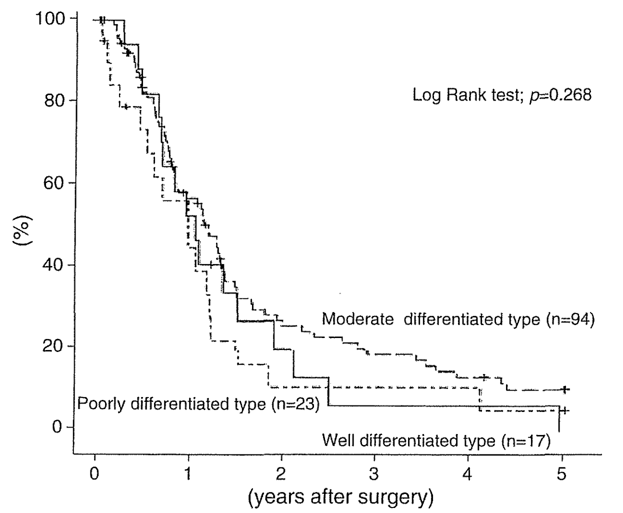


**Fig. 3** Overall survival for the patients with positive lymph node metastases based on the histological differentiation type. Among the patients diagnosed as being positive for lymph node metastases there were no significant differences in the survival rates between the histological tumor differentiation types

The prognosis for patients with ICC after resection is still unsatisfactory. The 5-year overall survival rate has varied from 4.1 to 43% [1–11]. From our present data, the median survival time was 20 months, with 3- and 5-year survival rates of 36.4 and 29.2%, respectively. The subgroup of patients with negative lymph nodes ($n = 141$) showed an actuarial 5-year survival rate of 48.6%. The main prognostic factors for ICC have recently been investigated in several surgical series [7–11]. The factors identified by these studies include a positive surgical margin and the presence of intrahepatic metastases, vascular invasion, lymph node metastases, and periductal neural invasion [1–10]. Our present study has demonstrated that the prognostic factors for survival after liver resection of ICC are a high serum CA 19-9 level (>135 U/ml), the presence of a positive surgical margin, lymph node metastases, and multiple hepatic tumors. Previous studies found CA19-9 expression to also be common in patients with ICC [17, 18]. In addition, previous analyses found CA19-9 positivity to be significantly associated with gender, age, tumor size, and cirrhosis, while logistic regression analysis indicated that the expression of CA19-9 was significantly associated with cirrhosis and lymph node metastases [19]. The surgical margin status has been reported to be a significant independent prognostic factor by several authors [4–10]. For example, Nakeeb et al. [20] cited 5-year survival rates of patients with negative surgical margins and those with positive surgical margins as 57 and 0%, respectively, and surgical margin status was a significant independent predictor for long-term survival in ICC. A different group reported that the overall survival after resection showed a significant correlation with the absence of tumor at the surgical margin, while a positive surgical margin was a significant independent predictor of poor prognosis by multivariate analysis [19–21]. Indeed, the multivariate analysis performed during our study revealed the surgical margin status to be a significant independent predictor for the overall patient survival. In cases of ICC with positive surgical margins, adjuvant chemotherapy with radiation may therefore be needed.

The value of lymph node dissection in ICC is still unclear; no clear guidelines on lymph node dissection exist. In addition, controversy remains regarding the value of lymphadenectomy for ICC [22, 23]. Lymph node dissection is rarely performed for ICC patients in Western countries. DeOliveira et al. [18], who have presented the largest series of resected cholangiocarcinomas in a single institute, mentioned that the harvesting of lymph nodes during the resection of intrahepatic bile duct cancers was not done routinely. Similarly, Tamandl et al. reported that extended lymphadenectomy was not routinely carried out beyond the hepatoduodenal ligament at their institution [11]; however, we found that our 3-year overall survival was comparable to that associated with other more extensive approaches reported in the previous literature [22] (35% in the present cohort vs. 36%). Routine lymphadenectomy around the hepatoduodenal ligament, peripancreatic nodes, celiac trunk, and lesser gastric curvature is commonly performed at several hepatobiliary centers [23, 24]. Nakagawa et al. [19] reported that a curative resection with lymphadenectomy improved the survival of patients with a solitary tumor and no more than two positive lymph nodes, and it has been reported that 6 of 23 patients who

**Table 7** Background of the 7 patients with nodal involvement who survived for more than 4 years

| Age (years) male/female | Operation method | Macroscopic type | Number of tumors | Tumor size (mm) | Surgical margin | Histological differentiation | Recurrence+ (region)/− | Outcome | Lymph node metastases | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | Positive no./total no. dissected | Distribution (no.)[a] |
| 62F | Partial hepatectomy (S7, 8) | MF | 2 | 45 | Negative | Poorly | + (lymph node) | 4.1 years, death | 1/not counted | n1(1) |
| 51F | Right trisectionectomy<br>Vena cava resection/construction<br>Biliary tract reconstruction | MF | 1 | 80 | Negative | Moderate | + (lymph node) | 4.2 years, alive | 2/5 | n1(2) |
| 63M | Left trisectionectomy<br>Biliary tract reconstruction | MF | 1 | 30 | Negative | Moderate | + (local) | 4.3 years, death | 2/12 | n(1)n2(1) |
| 40M | Left hepatectomy<br>Biliary tract reconstruction | MF + PI | 1 | 15 | Negative | Well | + (liver, lung) | 4.9 years, death | 3/3 | n(1)n2(2) |
| 72F | Right trisectionectomy<br>Biliary tract reconstruction | MF | 1 | 45 | Negative | Poorly | − | 5.8 years alive | 1/not counted | n1(1) |
| 58M | Left hepatectomy<br>Biliary tract reconstruction | MF | 3 | 100 | Negative | Moderate | + (lymph node) | 6.8 years, death | 3/24 | n1(1), n3(1), n4(1) |
| 59F | Left hepatectomy<br>Portal vein resection/construction<br>Biliary tract reconstruction | MF + PI | 1 | 25 | Negative | Moderate | − | 7.2 years alive | 2/not counted | n1(2) |

*MF* mass-forming type, *PI* periductal-infiltration type

[a] *n1* lymph nodes within hepatoduodenal ligament, *n2* lymph nodes along the common hepatic artery and retropancreatic lymph nodes, *n3* lymph nodes along the celiac axis and left gastric artery, *n4* paraaortic lymph nodes